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THE GENERAL HISTOLOGY AND TOPOGRAPHIC MICROANATOMY OF *AUSTRALORBIS GLABRATUS*

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WITH EIGHTEEN PLATES

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No. 3 — *The General Histology and Topographic Microanatomy of Australorbis glabratus*[1]

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INTRODUCTION

In a series of investigations on the potentialities of biological control of schistosomiasis, our efforts thus far have been concentrated on the accumulation of fundamental biological data on the snail vector, *Australorbis glabratus*. It was early realized that a knowledge of the normal histology of this snail was essential in order to permit a recognition of specific pathological alterations which might be induced by microorganisms. The present study was undertaken to satisfy this objective.

The gross anatomy of *A. glabratus* has been described by Baker (1945), and Paraense and Deslandes (1955). However, information on the histology of this snail is sparse (Faust and

Hoffman, 1934; von Brand and Files, 1947; Marcuzzi, 1950; Paraense and Deslandes, 1955) and inadequate as a basis for studying pathological changes. Despite the fact that the parasitic trematodes of man are known to utilize at least one fresh-water molluscan intermediate host, we have not discovered a comprehensive study of the histology of a single important molluscan host. The paucity of knowledge of the normal histology of fresh-water snails has been recognized recently by workers in this field, and some papers dealing with the digestive and genital tracts of a few fresh-water snails (Holm, 1946; Carriker and Bilstad, 1946; Abdel-Malek, 1954 a, b) have appeared.

The excellent monograph by Baecker (1932) on the micromorphology of *Helix* and other pulmonates, proved to be exceptionally helpful in the present studies.

MATERIAL AND METHODS

Laboratory-raised *Australorbis glabratus* of Puerto Rican origin were used. Our colony was started from stocks sent by Dr. Redginal I. Hewitt of the Lederle Laboratories, New York, and Dr. Donald V. Moore then at New York University. Although snails of various sizes were used, the descriptions given here are based mainly on specimens measuring between 9 and 20 mm. in size.

Snails were removed from the shell by crushing gently between two glass slides. The shell fragments were then separated with fine forceps under a dissecting microscope. With practice, intact snails could usually be obtained readily in this manner. The shell-free snails were immediately fixed either in a coiled or stretched position. A small amount of fixative was injected into the mantle cavity, employing a 27-gauge needle attached to a tuberculin syringe to insure rapid fixation of internal organs. Air in the mantle cavity was always removed by gentle manipulation under a dissecting microscope.

Zenker's or Maximow's fixative gave the best results for cytological study but hardened the genital tract and certain genital organs considerably. Formic acid Bouin's or Bouin's mixture had less tendency to harden the tissue, but the cytological structures were less well preserved. A solution of 5 per cent formic

acid in Zenker's stock was tried with results equal to those with Zenker's. Carnoy's and Newcomer's mixtures and 10 per cent neutral formalin were used for special purposes. Fixation was considered complete after 4 to 48 hours with aqueous mixtures, and after 3 to 6 hours with alcoholic solutions. Tissues were imbedded in paraffin (M. P. 56° C — 58° C) at 60° C and sectioned between 6 and 13 microns. Since it was necessary to remove all of the sand grains in the digestive tract for complete serial sections, the method of Carriker and Bilstad (1946) was used to obtain sand-free snails.

Lillie-Mayer's, Delafield's, Mallory's and Heidenhain's hematoxylins were used for nuclear staining, and eosin Y or phloxine B were employed as counter stains. Maximow's hematoxylin-azure II-eosin in thin sections gave excellent results for cytological studies. Special staining techniques used for the identification of various tissue components or structures were: Mallory's and Gomori's trichrome stains for connective tissue elements; Wolbach's Giemsa stain variant; Feulgen reaction for deoxyribonucleic acid; aldehyde fuchsin and acid orcein for elastic tissue; Hotchkiss' (1948) periodic acid-Schiff (PAS) for carbohydrate; thionin (at pH 4.0 to 5.0) and toluidine blue 0 for metachromatic substance; Bielshowski-Glees' and Bodian's silver impregnation; Korson's technique (1951) for nucleic acids; alcian blue 8GS (Steedman, 1950) for acid mucopolysaccharides; and Millon's reaction (Pearse, 1953; Lillie, 1954).

In the course of this histological study, two types of microorganisms — an acid-fast bacillus and a yeast-like organism — were encountered. Both organisms were always found associated with histopathologic changes (Pan, 1956). Since these histopathologic changes frequently aided in identifying the normal histologic structures of *A. glabratus*, reference will be made to the above-mentioned two microorganisms in the later sections.

The terminology used to designate various organs is based on Baker's monograph (Baker, 1945) on planorbid snails. The measurements of snails are given in shell diameter and thickness.

DESCRIPTION
TOPOGRAPHIC MICROANATOMY

Since a knowledge of the topography of various organ systems is necessary for the description of the histology of *Australorbis glabratus,* a brief account of its topographic microanatomy is given. One longitudinal and four cross-sections were selected to show the relations of various organs. Although the sections were selected on an arbitrary basis, it is believed they provide an adequate perspective of the microanatomy of the organ systems. The photomicrographs of these sections are self-explanatory, and, therefore, only important landmarks are indicated.

Figure 1 is an approximate median longitudinal section of a stretched snail measuring 15.0 x 5.1 mm. Figures 2 to 5 were taken under higher magnification and represent four areas of Figure 1. Figure 6 is a cross-section through the ganglion ring, and Figure 7 is through the spermatheca; these were from the same snail which measured 12.8 x 4.0 mm. Figure 8 is a cross-section through the stomach of a snail (10.7 x 4.1 mm.), and Figure 9 is through the overlapping area of the liver and ovotestis of a snail measuring 20.0 x 6.5 mm.

The important landmarks which should be noted in Figures 1 to 9 are to be found in the appropriate captions.

HISTOLOGY

Baecker (1932) divided the various systems of land pulmonates into two major categories, tissue (*Gewebe*) and organ (*Organe*), and made several subdivisions for each category. This classification is logical but has considerable overlapping. The following classification of the various tissues and organ systems of *Australorbis glabratus* was adopted in the present study:

I. Epithelium
II. Connective Tissue
III. Muscular Tissue
IV. Nervous System and Sensory Organs
V. Circulatory System
VI. Respiratory System
VII. Renal Organ
VIII. Alimentary System
IX. Reproductive System

I. Epithelium

Anatomically the epidermal tissue can be divided into three major zones: (a) that exposed to the outer world, including the head-foot organ and the mantle collar, (b) that always protected by the shell, including the mantle, columellar muscle, liver and ovotestis, and (c) the mantle cavity surface (respiratory surface). Baecker (1932) demonstrated that the epidermal tissue in land pulmonates consists only of a simple epithelium over the body surface. This is also the case in *A. glabratus* except in one small area at the dorsal rim of the mantle collar where there is pseudostratified columnar epithelium (Figs. 1 e, 11). The epidermal sheet rests on a basement membrane which is supported by a layer of connective tissue containing various amounts of smooth muscle fibers. The coelomic cavity is lined incompletely with flat cells.

Epithelium covering the exposed body surface. The surface of the foot or the sole is covered with a layer of tall columnar epithelial cells (*ca.* 22.5 microns in 18.8 x 6.5 mm. snail) possessing oval to elongated vesicular nuclei, relatively rich in chromatin. The free surface of these cells is heavily invested with long cilia. A row of basal bodies, each of which is connected with a cilium, lies immediately beneath the plasma membrane. The basement membrane can be seen clearly in sections cut at right angles and stained with Gomori's, Mallory's trichrome, or PAS. The epidermal cells on the foot surface appear to rest on the basement membrane with their short processes embedded therein. The cytoplasm of these cells is granular and lightly basophilic. The nucleus is located between the middle and basal third of each cell (Fig. 10). The cilia become sparse and disappear over the side wall of the head-foot organ where the tall, columnar, simple epithelium of the foot surface is replaced by low columnar-to-cuboidal simple epithelium. The epidermal cells of the mantle collar are also of this type except in a small area at the dorsal rim where there is pseudostratified columnar epithelium (Fig. 11). The cytoplasm of these cells is granular, moderately basophilic, and contains yellowish-brown pigment. This pseudostratified epithelium has a glandular appearance like that of the prointestine and may prove to have functions connected with shell regeneration.

Epithelium covering the area usually protected by the shell. This area includes the mantle surface, most of the columellar muscle, the liver and ovotestis (Fig. 1). The epithelial cells are characterized by having a dense deposit of brownish-black pigment in the cytoplasm, which usually obscures cellular structures. This pigment, named melain by Simroth in 1903 (Baecker, 1932), is not seen in the other two zones. Since this pigment stains green with Giemsa or thionin and reduces ammine silver, it is probably related to melanin of higher animals. The epithelial cells covering the mantle are low columnar to cuboidal with parabasal, round nuclei (Fig. 12). These cells are transformed into flat or squamous epithelial cells in the region of the liver, ovotestis and columellar muscle. The basement membrane and supporting connective tissues are delicate. The nuclei of these cells contain fewer chromatin granules than do those of cells covering the head-foot area.

Epithelium covering the mantle cavity. Three types of epithelium can be recognized in the mantle cavity. These are flat cells, cuboidal cells without cilia, and columnar cells which may be ciliated. The flat cells cover the surface of the wall of the coelomic cavity. The rest of the mantle cavity surface, except the surface of the three ridges (rectal, dorsal and kidney ridges), is covered with a sheet of cuboidal epithelial cells with round, parabasal nuclei (Fig. 13). The rectal, dorsal and kidney ridges are covered with a sheet of columnar cells that are very tall at the summits of the ridges and are provided with dense cilia in this area. These tall cells measure 30 microns in height in a snail of 12.8 x 4.0 mm. Unlike those of the foot surface, they rest on the basement membrane without anchoring short processes. The nuclei are oval, rich in chromatin and located in the middle third of the cytoplasm. The granular cytoplasm has three zones, the surface and the basal zones staining acidophilic, and the middle zone basophilic. Cilia arise from basal bodies located beneath the plasma membrane. The nuclei of the cuboidal cells are spherical and located near the basement membrane. The granular cytoplasm of the flat and of the cuboidal cells stains lightly basophilic throughout and these cells lack cilia. These two types of cells also have a smooth basal surface where

it is in contact with the basement membrane. Mucus-secreting cells (goblet cells) are scattered among the columnar and cuboidal cells and secrete mucus through the interstices between epithelial cells. Pigment does not occur in the epithelial cells in the mantle cavity.

II. Connective Tissue

The connective tissue of *A. glabratus* occupies the region between the organs and tissues and forms a membranous covering or sack enclosing the organs in the body cavity. The connective tissue contains various cellular and fibrous components and may be classified as to type depending on the relative proportion of the individual components. Baecker (1932) recognized four cellular components (*Fibroblasten, Blasenzellen, Körnchenzellen,* and *Pigmentzellen*) and two fibrillar components (*praekollagene Fibrillen* and *Gitterfasern*) in the connective tissue proper of land pulmonates. In *A. glabratus* the cellular components consist of: (a) fibroblasts, (b) pigment cells, (c) vesicular cells, and (d) mucous cells, and the fibrous components consist of collagenous-like fibers and delicate fibrils on the muscle fibers (*Gitterfasern* of Baecker).

Two distinctly different types of connective tissue can be recognized. These are loose "vascular" connective tissue and dense connective tissue. Between these extremes, intermediate forms occur. The loose "vascular" connective tissue (Fig. 14) is characterized by an open network of slender fibroblasts which, in section, appear as numerous oval or irregularly round perforations, the "*Zirkulationslücken*" of Kisker (1923). These perforations, or circulation spaces, hold the hemolymph of the snail, which stains a homogeneous pink with eosin Y (Fig. 14 a). Few additional cellular and fibrillar elements are present in this type of connective tissue. So-called "concretions" occur in the meshworks. These are crystalline-like structures of irregular shape and size (Fig. 14 c) and stain light blue with hematoxylin or light pink with PAS. The loose "vascular" connective tissue is found characteristically in the dorsal wall of the coelomic cavity, in the pseudobranch, the liver and the ovotestis (Figs. 6, 7, 9).

The dense connective tissue contains numerous fibroblasts, pigment cells and some amoebocytes as well as large amounts of collagenous-like fibers. There is no fibroblastic meshwork, but the cellular and fibrillar elements are embedded in the ground substance to form a compact tissue mass. Smooth muscle fibers are also abundant in this type of connective tissue. Since the ground substance, fibroblasts and collagenous-like fibers all stain pinkish blue in hematoxylin-eosin preparations, the ground substance may obscure the cytoplasm of the fibroblasts and also the collagenous-like fibers. *"Zirkulationslücken"* are present, but they are small in size and number and are inconspicuous. The dense connective tissue is seen characteristically in the foot proper and in the core of the tentacles (Fig. 15). Staining of this tissue with aldehyde fuchsin and acid orcein yielded negative results; therefore, as in land pulmonates (Baecker, 1932), *A. glabratus* is without true elastic fibers.

Fibroblasts. The fibroblasts of *A. glabratus* appear to have the ability to transform into a variety of cell types and to be involved in the repair of damaged tissues. In section they are fusiform or spindle-shaped but may have several branching processes (Fig. 16). These processes attach to or end on nearby fibroblasts and form the meshwork of the loose "vascular" connective tissue. The elongated or oval nucleus of the fibroblast has a delicate nuclear membrane, one or two nucleoli and moderately rich, fine chromatin. In stained material, the cytoplasm usually appears to be scanty and can be visualized only at the poles of the nucleus. It stains an almost homogeneous, light pinkish blue in hematoxylin-eosin preparations.

Various amounts of brown to brown-black pigment are frequently seen in the fibroblasts; and since intermediate forms may be seen, especially in pathological tissue (Fig. 17), it is considered that pigment cells may be derived from fibroblasts. The possible transformation of fibroblasts into amoebocytes will be described later.

Pigment cells. These cells approximate 15 x 21 microns (Fig. 17). They are very irregular in shape, being round, oval or elongated, and may bear processes, as described for this type of cell in land pulmonates by Baecker (1932). The nucleus is also irregularly shaped, may be round, oval or lentiform and is

frequently eccentric with relatively rich chromatin. The cytoplasm contains acidophilic granules which may be obscured or completely replaced by brown to brown-black coarse pigment granules. These pigment granules react to various stains similarly to the pigment of the epithelial cells. While pigment cells are distributed throughout the connective tissue, they are especially abundant in the rectal ridge and the renal ridge, and in pathological tissues. They may derive from fibroblasts as described in the preceding section. Their presence in large numbers was frequently connected with certain microbial infections (Pan, 1956). Marcuzzi (1950) described the pigment cells in *A. glabratus* as being excretory in function. Stein and Mackin (1955) reported that increased numbers of pigment cells, in oysters, were associated with certain infections.

Vesicular cells. Baecker (1932) described and discussed vesicular cells (*Blasenzellen*) in land pulmonates. Carriker and Bilstad (1946) found them in *Lymnaea stagnalis appressa*. These cells are irregular in shape, being round, ovoid, elongate and spindle-form, and measure up to 70 x 35 microns (Figs. 18, 52). The nuclei are relatively small, quite regular in shape, usually round or oval, and contain few chromatin granules. Binucleated cells are seen frequently. The nuclei are often eccentric and may be attached to the cell membrane. The cell membrane usually is discrete and stains well with eosin, aniline blue or fast green. The characteristic homogeneously-staining cytoplasm may contain a fibrillar network which stains less intensely than the plasma membrane, and reacts strongly with PAS suggesting the presence of carbohydrate material. In hematoxylin-eosin preparations the cytoplasmic mass may appear condensed around the nucleus, thus creating a space in the cytoplasm. The vesicular cells are distributed in many parts of the body; they form small groups in the radular carrier and the dorsal wall of the buccal cavity (Figs. 49, 52). The connective tissue of the liver and ovotestis also contains many of these cells.

Schaeffer (Baecker, 1932) postulated that the vesicular cells serve primarily as elastic supporting structures due to the fluid contents enclosed in the thick cell membrane. Since they occur in large numbers in the radular carrier and in the dorsal wall of the buccal cavity, they appear to have this supportive function.

However, the large amount of PAS-positive material also suggests other functions, such as the storage of carbohydrates. This latter function may be especially important in the connective tissue of the liver and ovotestis where it is probably connected with digestion and gametogenesis respectively, as suggested by Faust (1920).

Mucous cells. Scattered, well-developed mucous cells occur in small numbers throughout the connective tissue proper in *A. glabratus.* They are also found concentrated in large numbers in two glands: (a) a foot gland and (b) a buccal gland. The buccal gland will be described in the section on the alimentary system. Unlike the land pulmonate (Baecker, 1932), the foot gland of *A. glabratus* does not have an excretory duct. Each mucous cell comprises a secreting unit and secretes mucus through a gradually tapering process (Fig. 19). The cell is usually teardrop or retort-shaped but may be pleomorphic. In size these cells vary considerably but usually measure about 23 microns at the widest diameter. The relatively round or oval nucleus is extremely rich in chromatin and possesses an eccentric, large nucleolus. When the cells are filled with secretion materials, the nuclei tend to lose their normal structure and become pyknotic. As has been described by Baecker (1932) and Carriker and Bilstad (1946) in other species, these cells contain various cytoplasmic structures depending on the stage of secretory activity. In hematoxylin-eosin preparations, the cytoplasm of actively secreting cells is filled with large basophilic granules which usually obscure the nucleus. These basophilic granules are strongly PAS-positive but do not show metachromasia with thionin or toluidine blue O. They stain with orange G in Mallory's trichrome stain. After the secretory materials are released, the cytoplasm loses the basophilic substance, becomes finely reticular, and shows only weak reaction to PAS stain. In the foot gland, several of the mucous cells form subgroups, and the secreting processes bundle together and extend toward the epithelial sheet. The muscle fibers of the foot run through and between the subgroups. These muscles probably serve to force the secreted mucus through the intercellular spaces of the epithelial cells.

Collagenous-like fibers. The connective tissue fibers of land pulmonates resemble the collagenous fibers of higher animals (Baecker, 1932) and are described as collagenous-like fibers from *Lymnaea stagnalis* by Carriker and Bilstad (1946). In *A. glabratus* these fibers are more delicate than the muscle fibers, and are differentiated poorly in hematoxylin-eosin preparations taking a light pinkish-blue hue. They are colored blue by Mallory's triple stain and green by Gomori's trichrome, but they are not impregnated by Bielschowski-Glees' or Bodian's silver stain. They are more abundant in dense connective tissue than elsewhere. Fibers seen in the basement membrane of epithelial sheets are more delicate than the collagenous-like fibers in the connective tissue proper. The staining reaction of both types is similar.

Delicate fibrils on the individual muscle fibers. Baecker (1932) described delicate fibrils (*"Gitterfasern"*) lying on the surface of sarcolemma of land pulmonates; these can be stained with aniline blue or by silver impregnation. The perimycium of *A. glabratus* contains similar fibers which encircle the muscle fibers. Although they stain with aniline blue in Mallory's trichrome, silver impregnation fails to stain them. They are extremely delicate and recognizable with difficulty unless special stains are employed. From the staining characteristics, these fibrils are probably modified collagenous-like fibers.

III. Muscular Tissue

It has been pointed out by Olson (1942) that although the muscle fibers of the molluscs have been much studied, there is yet no real agreement as to their structure. Baecker (1932) summarized the work of previous investigators and stated that two types of muscle fibers, smooth and striated, are present in land pulmonates, and that the latter is a very primitive type. Marcuzzi (1950) described both smooth and striated muscle fibers in *A. glabratus.* We observed three different types of muscle fibers in our preparations, but we have not been able to observe fibers which possess periodic striations comparable to those of higher animals. Baecker (1932) pointed out that

the muscle of land pulmonates does not react to stains exactly as that of the higher animals, and in this respect the muscle fibers of molluscs are regarded by him as somewhat related to collagenous-like fibers of connective tissue. This is also true in *A. glabratus*, in which muscle fibers, especially smooth fibers, may occasionally stain as do collagenous-like fibers. Thus, some of the smooth fibers or portions of smooth fibers may stain with fast green in Gomori's trichrome or with aniline blue in Mallory's triple stain instead of with Chromatrope 2 R or Orange G respectively.

The three types of muscle fibers observed in *A. glabratus* are (a) granular muscle or heart muscle, (b) intermediate granular muscle, and (c) smooth muscle.

Granular muscle or heart muscle. Marcuzzi (1950) reported that the heart muscle of *A. glabratus* is of the obliquely striated type of Plenk (1924). We could not demonstrate striations (cross, or oblique) in the muscle fibers of this organ by any of the ordinary cytological techniques (iron hematoxylin, phosphotungstic acid hematoxylin, toluidine blue O, hemalum, phloxine B and eosin Y). In cross-section the fibers appear round, oval, angular or irregular in shape. The fiber is enveloped by a delicate, somewhat refractile membrane (plasmalemma or sarcolemma) which stains lightly basophilic. The fibers measure ± 12 microns in thickness. No myofibrils could be recognized, but the fiber is filled with relatively coarse acidophilic granules. These granules are distributed quite evenly within the fibers, but may at times be concentrated in the peripheral zone (Fig. 20). Occasionally these granules are seen to be connected by very delicate fibrils.

In longitudinal sections (Fig. 20) the fiber is spindle-shaped with a thickened mid-portion at the site of the nucleus. The coarse acidophilic granules also are more or less concentrated in the peripheral zone. Myofibrils were not recognized in the longitudinal positions. The nucleus is single, round to oval in shape and has but little chromatin. There is no clear area around the nucleus, as occurs in vertebrates. The heart muscle fibers of *A. glabratus* anastomose freely to each other as in vertebrates, but there are no intercalated discs. Connective tissue is scarce, and the fibroblasts attach here and there on the muscle fibers.

The fibroblasts sometimes contain a small amount of brown granular pigment. There is no true endocardium, but the outer surface of the heart is protected by a well-developed epicardium (Figs. 33, 35).

This type of granular muscle fiber is seen only in the heart of *A. glabratus*. Infrequently the granules in some fibers may be so arranged that striation is suggested. Marcuzzi (1950) apparently observed this condition. The heart muscle of *A. glabratus* forms only primary bundles.

Intermediate granular type of muscle. This type of fiber is apparently an intermediate form between the granular muscle (heart muscle) and smooth muscle, and is confined to the buccal mass. It may measure up to 10 microns in thickness. In cross-section the muscle fibers appear more irregular in shape than in heart muscle and present round, oval, triangular and quadrangular outlines. There are two clearly defined zones: a rather heavily stained peripheral zone and a lightly stained central zone (Fig. 21). The refractile sarcolemma is thinner than that of the heart muscle. Delicate myofibrils are evenly packed in the peripheral zone and stain intensely acidophilic. The central zone contains a number of coarse granules which are stained less acidophilic than the myofibrils.

In longitudinal sections (Fig. 21) the fibers are spindle-shaped, having a slightly thickened middle portion. Myofibrils run parallel to the long axis of the fiber in the more heavily stained peripheral zone. The inner portion stains lightly acidophilic and contains evenly scattered, coarse granules which occasionally may be linked with fine threads. The granules are, as in the case of heart muscle, arranged at times so as to show an irregular, cross-striation-like appearance. This striated appearance is not constant and when it does appear in a preparation only a few such fibers show it. Each fiber contains one centrally-located nucleus at the thickened middle portion of the fiber. The nucleus is oval, poor in chromatin granules and usually possesses a nucleolus. This type of muscle fiber has been described for many molluscs by various authors, especially by Olson (1942) who studied it in the radular retractor of *Busycon* sp.

The muscle of the buccal mass forms primary and secondary bundles. Small amounts of endomysium bind individual fibers to form primary bundles which are in turn bound by perimysium to form secondary bundles. These interstitial tissues are collagenous in nature and can be stained with PAS, Mallory's or Gomori's trichrome stains. Fibroblasts may attach on the surface of sarcolemma and may contain brown pigment granules.

Smooth muscle. This type of muscle cell is comparable to that of vertebrates and constitutes the major musculature of *A. glabratus*. It is found in the columellar muscle, foot muscle, alimentary canal, genital tracts, tentacles, and other areas. Its fibers are the smallest of the three muscle types, measure less than 9 microns in thickness, and show considerable morphologic variation.

In cross-section the fibers appear round to ovoid or angular in shape. In longitudinal sections they are fusiform with a thickened mid-portion containing the nucleus. The fibers stain uniformly acidophilic and do not contain granules except rarely about the nucleus. Myofibrils are abundant and can be recognized readily running along the long axis of the fibers in properly fixed specimens (Fig. 22). They are distributed evenly in the fibers and are packed together tightly. A sarcolemma cannot be recognized as such, but a very thin, refractile outline surrounds the fiber.

The single nucleus is elongate or oval. It lies along the long axis of the fiber and is somewhat eccentric in position. There is usually a nucleolus; the chromatin granules are small and few. Dark brown pigment may be present around the nucleus, especially at its poles.

Most of the fibers in the foot run singly in various directions through the dense connective tissue. In the columellar muscle the fibers form primary and secondary bundles which run along the long body axis. The endomysium and perimysium are more abundant here than in the buccal mass musculature.

IV. Nervous System and Sensory Organs

The nervous system of *A. glabratus* consists of central ganglia, peripheral ganglion, individual ganglion cells, and nerves. The sensory organs observed in this study are: statocysts, eyes,

osphradium, tentacles, and five groups of small sensory cells, one each at the base of each tentacle and the margin of each lip respectively.

Central ganglia. According to Baker (1911 and 1945) and Baecker (1932) the central ganglion ring of pulmonate snails is characteristically composed of 11 separate ganglia which are linked by communicating branches of commissures to form a ganglion ring around the esophagus immediately behind its union with the buccal mass. There are two cerebral ganglia above the esophagus, and below the esophagus there are two buccal ganglia, two pedal ganglia, two pleural ganglia, two visceral ganglia and a single abdominal ganglion (Baker 1945).

The arrangement of the ganglia in *A. glabratus* is essentially the same as in other pulmonate snails. The paired visceral ganglia are not of equal size, the left ganglion being somewhat larger than the right one. All of the parallel ganglia and their commissures are covered by a common connective tissue sheath, the epineurium. Histologically, these ganglia are similar in arrangement and composition (Figs. 2, 6, 23). The epineurium is a relatively thick connective tissue layer which is rich in cellular and fibrous elements. The collagenous-like fibers are relatively coarse, loosely packed, and, for the most part, run parallel to each other along the long axis of the ganglion. A few cross fibers are also present. The cellular elements are similar to the fibroblasts of the connective tissue. Each ganglion is directly covered by a sheath, one cell thick, the perineurium. It stains more intensely than the epineurium with connective tissue stains such as Mallory's or Gomori's trichrome. Arteries and blood spaces are present in the epineurium. Vesicular cells seen in the epineurium of land pulmonates by Baecker (1932) were not observed in the ganglia of *A. glabratus*.

Ganglion cells (Figs. 6, 23, 24) are located on the periphery of the ganglia and many are in contact with the perineurium; however, areas where the nerve root and commissure leave a ganglion are devoid of ganglion cells. The center of the ganglion contains neurofibrils sent out by the ganglion cells and is thus filled with neurofibrils which run in various directions in bundles.

A few fibroblast-like slender cells occur among these neurofibrils in the ganglion and larger nerves. These cells under pathological stimuli may become hyperplastic and phagocytic and apparently are equivalent to the glia cells of the vertebrate central nervous system.

According to Nabia (Baecker, 1932) two types of ganglion cells are present in the gastropod nervous system; one, the so-called ordinary ganglion cell with a relatively large amount of cytoplasm, and the second, the chromatinic ganglion cell, with sparse cytoplasm and of small size. In *A. glabratus* the chromatinic ganglion cells form two prominent groups in the dorsoposterior corner of each cerebral ganglion. The nuclei of these cells, measuring 7 x 4 microns, are slightly larger than those of fibroblasts, and the cytoplasm is seen as a small rim around the nucleus. The so-called ordinary ganglion cells may measure 59.5 x 52.5 microns, with nuclei of 42.0 x 24.5 microns. The nucleus has a definite nuclear membrane, contains a large number of coarse chromatin granules held in a linin network, and one or two nucleoli (Fig. 24). The nucleolus is located eccentrically and may be oval. The cytoplasm contains a large amount of basophilic material in the form of coarse granules or small plaques (especially in thionin-stained preparations) which resemble Nissl's bodies in the vertebrates. Although silver impregnation (Bielschowski-Glees' and Bodian's method) was not successful in impregnating neurofibrils, many of the Bouin-fixed preparations, when overstained with hemalum, contained fibrillar networks in the cytoplasm of the larger ganglion cells. Neuroglia cells may surround the larger ganglion cells, but none of them was observed inside the cytoplasm of ganglion cells (i.e., the "*Trophospongien*" of Holmgren, 1905). The structure of the central ganglia, as described above, is somewhat similar to the central nervous system of higher animals in that the neurons are located in the peripheral zone (gray substance) and the neurofibrils are concentrated in the central zone (white substance). Baecker (1932) also pointed out this correlation in the central nervous system of land pulmonates. However, myelinated nerve fibers were not demonstrated in *A. glabratus;* neither were they seen in land pulmonates by Baecker (1932).

This author also described neuroglia cells in the ganglia of land pulmonates and observed that the cells were concentrated in the peripheral zone of the ganglia. In *A. glabratus*, neuroglia cells are similar morphologically to fibroblasts and are scattered evenly in the ganglia in small numbers (Fig. 23). Hyperplasia and transformation of neuroglia into phagocytes were observed in a few snails which were infected with an unidentified yeast-like organism. In some instances partial or complete replacement of ganglion cells and neurofibrils of affected ganglia by neuroglia was also observed (Fig. 25).

Peripheral ganglion cells and peripheral ganglion. Peripheral ganglion cells are scattered singly or in small numbers in various organs and tissues. These cells are morphologically similar to those in the central ganglia. There appears to be only one peripheral ganglion present in *A. glabratus*. This ganglion is associated with the osphradium (Fig. 29) and will be described later.

Nerves. The structure of the nerve in *A. glabratus* resembles that of the *Helix* nerve described by Baecker (1932). The bundles of neurofibrils are covered by the connective tissue sheath (peri- and epineurium) of the ganglion in the root area. The perineurium is lost shortly below the ganglion. The epineurium is continuous with that of the ganglion and is relatively thick in the larger nerves. In small peripheral nerves the epineurium becomes one cell thick and has only a few collagenous-like fibers. Most of the fibers run along the long axis, but circular fibers are also present. These stain light blue with Azan triple stain, green with Gomori's trichrome, and pinkish-blue with hematoxylin-eosin. The neurofibrils stain bluish-red and dark red with Mallory's and Gomori's trichrome respectively. Neuroglia cells are present among the neurofibrils and are more abundant in larger nerves. They are usually spindle-shaped and run with or obliquely to the long axis of the nerve (Fig. 26). No myelinated neurofibrils were observed in *A. glabratus*. The nerve trunks and large nerves of *A. glabratus* can be recognized without difficulty in hematoxylin-eosin preparations by their staining characteristics and structures. In cross-section of the larger nerves a connective tissue substance may form incomplete septa

at the periphery. Since no myelinated neurofibrils were demonstrated in *A. glabratus,* the nerve of this snail belongs to the "incomplete type" of Schultze (Baecker, 1932). The axis cylinders, or neurofibrils, in individual nerves, are embedded in a ground substance which takes up fast green or aniline blue.

Statocyst. The statocyst, or the balancing organ of the pulmonates, has been described in detail for the genus *Helix* by Baecker (1932). The statocyst in *A. glabratus* appears to have structures similar to those of *Helix.* In *Australorbis* it is a paired organ located in the latero-posterior corner of each pedal ganglion at the root of the commissure to the pleural ganglion. It is an oval-shaped sac measuring approximately 94.5 x 66.5 microns (20 x 6.4 mm. snail)and lies embedded in the epineurium of the pedal ganglion (Fig. 23). Unlike *Helix,* the main nerve to the statocyst of *A. glabratus* originates from the pedal ganglion, but it also receives a smaller nerve from the pleural ganglion. The wall of the statocyst consists of (1) an outer layer, (2) a middle layer and (3) an inner or epithelial layer. The outer layer is a connective tissue sheath, rich in circular collagenous-like fibers, and is fused into the epineurium of the pedal ganglion. The middle layer is a very thin homogeneous capsule which can be recognized only with a connective tissue stain. It is considered by Baecker (1932) to be the basement membrane of the epithelial layer in *Helix.* The inner or epithelial layer is composed of two types of epithelial cells which intermingle and form a membrane one-cell thick. One type of cell is small, flat and fibroblast-like with chromatin-rich nuclei but with little cytoplasm and indistinct boundaries. The other type includes cells which are half-moon in shape with the convex side on the basement membrane. They usually measure 25 x 12.5 microns and are the "giant cells" of Baecker (1932). The nucleus is small, oval, and is somewhat peripherally situated. On the basal side of the nucleus, the basophilic cytoplasm may contain large vacuoles supported by fibrillar structures. Neurofibrils may be traced into the cytoplasm of these larger cells but are not present in the smaller or first type. The free surface of the "giant cells" is covered with long, but sparse, cilia. The lumen of the statocyst sac contains ovoidal bodies, the statoliths. They measure 4 x 3 microns and stain lightly basophilic (Fig. 24). The peripheral zone appears denser than the central zone.

Eyes. The eyes of pulmonates are well developed and are comparable in structure to those of vertebrates. Smith (1906) made a detailed study of the eyes of pulmonates, and the more recent studies are summarized by Baecker (1932). According to the former author, land pulmonates have the following eye structures: optic capsule, cornea, retina, lens, vitreous humor, optic nerve and accessory retina.

The eyes of *A. glabratus* are located latero-posteriorly to the base of each tentacle and are embedded in the dorsal wall of the head. All of the above-mentioned eye structures for pulmonates, except the accessory retina, were recognized in *A. glabratus* (Fig. 27). In addition, a few delicate smooth muscle fibers were observed to be attached to the optic capsule.

The optic capsule consists of a connective tissue sheath which is one cell thick on the surface of the cornea, but becomes two to three cells thick near the optic nerve where it fuses with the epineurium or connective tissue sheath of the latter. The cornea and retina together form a closed sac which constitutes a wall, one cell thick, enclosing the lens and the vitreous humor. Between the cornea and the epithelium of the head there is a small space which is filled with tissue fluid and contains a few wandering cells or amoebocytes. The cornea is of non-pigmented, squamous epithelium and is in close contact with the lens on the inner surface and with the optic capsule on the outer surface. It covers approximately one-third of the surface of the eyeball.

The retina (Fig. 27) makes up the remaining portion of the sac. As shown for *Planorbis trivolvis* by Smith (1906), there are three differentiated regions in the layer of simple epithelium. The outer zone is in contact with the optic capsule and comprises the non-pigmented but nucleated portions of the retinal cells. The middle region consists of the constricted portions of the sensory cells and the pigmented portions of the pigmented or supportive cells. The sensory cells in this region are usually obscured by the heavily pigmented, thick cytoplasm of the pigment cells. The inner zone is made up of the rods of sensory cells and is in contact with the vitreous humor. Both types of retinal cells (the sensory and the pigment) are attached to the optic capsule by radiculae (Smith, 1906). The pigment cells are

more slender and basophilic than the sensory cells at the peripheral zone. The nuclei of the pigment cells generally are nearer the capsule and are smaller than are the nuclei of the sensory cells.

The rods constitute those portions of the sensory cells which extend beyond the middle pigment zone, and are light receptors according to Smith (1906). In the P.F.F.-fixed and hematoxylin-phloxine B-stained preparations, the rods appear as club-shaped objects surrounded by a radially striated thick mantle.

The lens is spheroidal, occupies most of the ocular sac, and is strongly acidophilic. The peripheral zone of the lens is homogeneous, but the central zone is porous, perhaps an artifact due to fixation and staining. The vitreous humor is very small in amount and stains lightly pink.

The optic nerve originates from the cerebral ganglion and has the structure of a medium-sized nerve.

Osphradium. Baecker (1932) did not describe the osphradium, or the so-called olfactory organ, as occurring in land pulmonates. The osphradium in *A. glabratus* is located where the mantle collar joins the neck of the snail between the median line and the pneumostome siphon (Fig. 1 f). It is a somewhat elongated pear-shaped sac, about 300 x 120 microns in a snail measuring 15 x 5.1 mm. The opening of the osphradium is obscure in living specimens. In section, the lumen is lined with a layer of tall columnar epithelial cells covered with long, dense cilia (Figs. 28, 29). Two types of columnar epithelial cells can be identified. One has a basal oval nucleus which is rich in chromatin, and the cytoplasm is filled with basophilic granules. The second type is a slender cell compressed between cells of the former type and has a central elongated nucleus. The cytoplasm is scanty and cannot be clearly recognized. The epithelium of the osphradium is replaced by the cuboidal cells of the neck and mantle surfaces at the opening to the exterior. The epithelium rests directly on a layer of smooth muscle fibers (mostly circular and some longitudinal) that contain a few fibroblasts (Fig. 28). The lower portion of the sac is surrounded by a peripheral ganglion (Fig. 29). The structure of this peripheral ganglion is similar to that of the central ganglia. Neurofibrils from these ganglion cells

appear to penetrate through the muscle layer and end on the epithelial cells. The ganglion attached to the osphradium receives a thick branch nerve from the left visceral ganglion.

Tentacles. There is but one pair of tentacles in *A. glabratus.* The tentacle is the most abundantly innervated and the most flexible structure of the snail. Thus, it is a very delicate tactile sense organ. A thick nerve trunk arises from the cerebral ganglion and, after reaching the root of the tentacle, passes through the central core of the tentacle to the very tip. Anatomically, the tentacle is a gradually tapering cylinder. On the cross-section it has a round contour except at the base where there is a leaf-like enlargement on the lateral side (Fig. 30). The core of the tentacle near the root consists of dense connective tissue which becomes less dense toward the tip. A central artery runs up the core to the tip where it empties into the peripheral blood sinuses. The connective tissue of the core sends out radial strands of fibroblasts to the epithelial sheet (Fig. 31). The nerve trunk is embedded in this core of connective tissue and also sends out many branches to the epithelium along the radial connective tissue bridges; the bridges also contain delicate muscle fibers. Longitudinal muscle fibers and pigment cells are abundant in the central connective tissue core. The hemolymph fills the interstices between the central core and the peripheral epithelium. The simple short columnar-covering epithelium bears dense cilia. The basement membrane is not distinct toward the tip. Beneath the basement membrane there are a few delicate circular muscle fibers intermingled with fibroblasts. Postulating from the histological structure, the extension of the tentacle is probably accomplished by the filling of the blood sinuses with hemolymph through the central artery. The retraction of the tentacle is apparently accomplished by the contraction of the longitudinal and circular muscle fibers with resultant emptying of the hemolymph from the sinuses.

Sensory cells at the margin of the lips and at the bases of the tentacles. There are five specialized groups of sensory cells, one associated with each leaf-like enlargement at the base of the tentacle and one at the margin of each lip. These cells are subdivided into many clumps by fibroblasts and are in close contact with the basement membrane (Fig. 32). They resemble the

chromatinic ganglion cells of the central ganglia. These sensory cells are short, fusiform in shape, contain chromatin-rich ovoid nuclei and have a very basophilic, vacuolated cytoplasm. One end of each fusiform cell fuses into the bundle of neurofibrils sent out from the branches of the nerve innervating the tentacle. The other end of the cell is directed peripherally and tapers off into a filament. Many of these cells are in direct contact with the epithelial cells of their respective areas. Although the exact nature of these cells is not clear, they appear to function as peripheral sensory cells.

V. Circulatory System

According to Baker (1945) the circulatory system of planorbid snails consists of a heart, arterial system, venous system, and blood sinus system. Histological description of these organs in land pulmonates is given by Baecker (1932).

Our histological studies of the circulatory system of *A. glabratus* fully agree with Baker's anatomical observations. However, the "loose vascular" connective tissue should be mentioned as an integral part of the circulatory system.

Heart. The heart consists of two chambers: a caudal pear-shaped, muscular ventricle, and a cranial pear-shaped, thin-walled atrium. These two chambers are joined at their wide bases by a constriction where there is a pair of muscular valves (Fig. 33). The valves are directed into the ventricle and are thin muscular sheets covered by cells resembling those of the epicardium. The junction between the ventricle and aorta is also provided with a thin muscular valve which is directed into the aorta (Fig. 34). The muscle of the heart is of the granular type already described.

The atrium has a very thin muscular wall and is more distensible than the ventricle. In sectioned material the lumen of the atrium is usually several times larger than that of the ventricle.

The thick muscular wall of the ventricle is formed by a three-dimensional mesh of branching and anastomosing muscle fibers, slightly more densely woven adjacent to the epicardium. The wall of the heart (both chambers) consists principally of longitudinal and circular muscle fibers. Some of the branching fibers

form trabeculae that cross the lumen to the opposite wall. Fibroblast-like, fusiform cells occur among the muscle fibers and are especially prominent in certain pathological conditions. In *Helix,* Baecker (1932) regarded these fusiform cells as perimysium and not as endocardium. There appears to be no true endocardium in *A. glabratus.*

The outer surface of the heart is covered with a continuous layer of cells which comprise the epicardium (Figs. 33, 35). These cells are round to short, columnar in shape, with distinct boundaries when the heart is contracted, but become flat and without clear boundaries when it is distended. In the contracted position of the heart, the nuclei of the epicardial cells are round to oval, subbasal in position and poor in chromatin granules. The cytoplasm is finely or coarsely vacuolated and contains basophilic filamentous material (Fig. 35). The structure of the epicardium appears to be adapted for great distention.

The heart is enclosed in the pericardial sac which is connected to the lumen of the saccular portion of the kidney by a renopericardial canal (see section on kidney) (Fig. 46). The pericardial sac is bordered by a portion of the saccular kidney, part of the mantle and by membranous tissue which is an extension of the mantle into the mantle cavity. The internal surface of the sac is lined by a sheet of flat cells which contain a moderate amount of dark brown pigment.

Arteries. In large arteries, including the aortae, the wall is composed of three layers (Figs. 36, 37). The lumen is lined with a layer of fibroblast-like cells which are very thin and barely recognizable under most conditions. Baecker (1932) stated that these lining cells as well as the connective tissue cells of the heart, are not truly endothelial in nature. From our observations in *Australorbis,* these cells may undergo hyperplasia and hypertrophy under pathologic stimuli; they then may become detached and transformed into phagocytes (Figs. 36, 40). Therefore, we believe these lining cells bear endothelial properties. Beneath the lining cells there is a layer of smooth muscle fibers, most of which are circular, but longitudinal fibers are also present. The muscular layer is covered with a varying amount of connective tissue. In small arteries the muscle and connective tissue layers may be absent, but the lining cells are well defined.

Veins. The veins have no definite wall and are tissue spaces which are lined incompletely with fibroblast-like cells (Fig. 38); thus the veins cannot be differentiated from the surrounding tissues. The veins connect freely with the blood sinus system.

Blood sinuses. The blood sinuses are tissue spaces which are interlaced abundantly by fibroblasts, some of which form a trabecula-like support. The most conspicuous blood sinuses are those in the mantle (Fig. 39).

The blood or hemolymph appears to be forced into the "loose vascular" connective tissue via arteries, and after bathing the organs and tissues is collected in the blood sinuses. It is finally returned to the atrium of the heart via the pulmonary and renal veins. Both of these veins pass along the lateral sides of the kidney and unite near the blind end of the saccular portion of the kidney before entering the atrium of the heart. The hemolymph in the "loose vascular" connective tissue is apparently squeezed forward by the smooth muscle fibers present in that tissue, as described by Baecker (1932). Our description of the vascular system of *A. glabratus* essentially agrees with that given by Baecker for land pulmonates. However, hyperplasia and transformation of the lining cells into amoebocytes are probably noted for the first time in the pulmonate snails.

Amoebocytes or wandering phagocytes. In *Helix*, Baecker (1932) described nucleated blood corpuscles which he regarded as equivalent to the leucocytes in higher animals; he called these nucleated blood corpuscles "*Amoebocyten*" and these were said to have phagocytic functions. In *A. glabratus,* only nucleated blood corpuscles occur and these cells closely resemble the amoebocytes described by Baecker in *Helix* (Fig. 40). Several kinds of amoebocytes have been described in molluscs by various workers (George and Ferguson, 1950; Wagge, 1955). Although there may be different types in *A. glabratus* we did not attempt their differentiation, and the nucleated cellular components of the hemolymph are all included as amoebocytes. They occur in small numbers in the circulatory system as well as in connective tissue. Because of the semi-open circulatory system of *A. glabratus*, the amoebocytes are normally fairly evenly distributed in the connective tissue, but in certain pathological states a large number may localize in diseased tissue areas (Fig. 41). In one type of

inflammatory reaction observed, a large number of microorganisms were present in the cytoplasm which then became rounded and enlarged from two to three times its normal size (Fig. 42). In sections, the amoebocyte of *A. glabratus* measures approximately 9 x 12 microns, but there is considerable variation in size and shape. However, many are round or oval with occasional lobose pseudopodia. The nucleus is vesicular, and round, oval or lentiform. It contains a moderate amount of coarse granular chromatin and is usually eccentric in position (Fig. 40). The cytoplasm is lightly basophilic and granular and usually shows coarse vacuolation.

Hemopoietic tissues. According to Baecker (1932), the origin of the amoebocyte in land pulmonates is unknown. However, recent workers have observed that the fibroblasts and epithelial cells of the mantle in *Helix* sp. transform into amoebocytes under certain conditions (Haughton, 1934; Crawford and Barer, 1951; Wagge, 1951, 1955). No mention of specially differentiated hemopoietic tissues was made by these workers. Haughton (1934) indicated that the blood vessels in the invertebrates were the place of origin of the amoebocytes. Our observations suggest that possible normal sites of production for amoebocytes are the blood sinuses and the wall of the saccular portion of the kidney which forms part of the pericardial sac. The fibroblasts which form the trabecula-like supports of the blood sinuses in the mantle frequently round up, but remain attached to the wall of the sinus by a cytoplasmic process. The typical elongated nucleus of the fibroblast is lost in this transition form and becomes vesicular as in the mature amoebocyte. The wall of the saccular portion of kidney bordering the pericardial cavity is composed of primitive tissue probably of mesenchymatous origin (Fig. 43). The cellular components are round or oval in shape but with irregular outlines and with processes which join with those of the neighboring cells to form a cellular reticulum. They are fairly closely packed and imbedded in a ground substance which contains some collagenous-like fibers. The cytoplasm is lightly basophilic, may contain several vacuoles, and in general closely resembles that of the mature amoebocyte. The nuclei are vesicular and also resemble those of amoebocytes. Mitotic figures are frequently seen in this tissue. The cellular reticulum has numerous blood spaces

which are connected with the blood sinuses of the saccular portion of the kidney. The appearance of this tissue resembles closely the medulla of lymph nodes in the vertebrates. Extreme hyperplasia in this tissue was noted in certain pathological conditions and the cellular components were observed to contain many microorganisms. Amoebocytes free in the blood spaces were regularly seen in this tissue. From its histological structure as well as its behavior in pathological states, this tissue may be regarded as hemopoietic tissue or lymphoid tissue, and it is described for the first time in this study.

In pathologic conditions, the fibroblasts in various parts of the body of *A. glabratus,* especially the rectal ridge and the kidney ridge, were also observed to participate in production of amoebocytes. Under such circumstances transition forms are frequently noted in the connective tissue.

Although the amoebocyte has been described by various authors as being concerned with digestion and with the repair of damaged shells (Yonge, 1946; Wagge, 1951; George, 1952; Wagge and Mittler, 1953), we found little activity of amoebocytes in *A. glabratus* which may be correlated with these functions. We observed few amoebocytes around the digestive tract of this snail. The main activity of the amoebocytes in *A. glabratus*, according to our observations, appears to be phagocytosis of foreign bodies, especially microorganisms.

VI. Respiratory System

The respiratory system of *A. glabratus* consists of a pneumostome equipped with a pneumostome siphon (pseudobranch), and the mantle cavity. The pneumostome is surrounded by a portion of mantle collar and the neck. It is loosely divided into two openings by the pseudobranch which protrudes out of the mantle cavity. The pneumostome siphon occupies the right opening and is actually a curled, small flap of tissue arising from the neck and possessing a moderately loose vascular connective tissue, as stroma. The epithelial cells of the mantle collar and the siphon are cuboidal to short columnar in type. In the stroma of these structures, pigment cells and smooth muscle fibers are present in moderate amounts.

The wall of the mantle cavity is provided with three ridges which protrude into the lumen and apparently serve to increase the respiratory surface. These are the kidney ridge, rectal ridge, and the dorsal ridge which is situated between the kidney ridge and the rectal ridge on the left side. The first two ridges contain two veins each and the dorsal ridge contains one vein. In each case, the veins run along the long axis of the ridge. Although the epithelium in the mantle cavity serves as respiratory epithelium, no special differentiation of these cells from the other, non-respiratory epithelia could be recognized. The cilia of the tall columnar epithelial cells on the summits of the three ridges apparently serve to guide the flow of water which enters the mantle cavity. The three types of epithelial cells of the mantle cavity surface have been described earlier. Since the supporting connective tissue underlying the basement membrane of the area between the ridges is very thin and the epithelium is therefore in close contact with the blood sinus system, the cuboidal epithelial covering of this area probably has an active respiratory function. Baecker (1932) described this form of respiratory cell in land pulmonates, but the tall ciliated columnar epithelial cells on the summit of the ridges were not mentioned. A few delicate smooth muscle fibers are present beneath the basement membrane. In addition to the veins in the ridges, the mantle is supplied with a bloood sinus system (Fig. 38) which communicates freely with the venous system and is also in intimate contact with the respiratory epithelium.

The three ridges frequently have been observed to be invaded by microorganisms with resulting hyperplasia of fibroblasts and amoebocytes.

VII. Renal Organ

Baker (1945) described the kidney of the Planorbidae as consisting of a small upper saccular portion, and elongated lower tubular portion, and a short ureter curving nearly 160° to the left before it opened into the mantle cavity at the pneumostome. Baecker (1932) considered the tubular portion of the kidney in land pulmonates as a secondary ureter, while Abdel-Malek (1952) held that both portions of the kidney in Planorbidae are similar

histologically but different anatomically. In *A. glabratus* the two portions of the kidney are histologically and anatomically distinct.

Tubular portion of the kidney. The characteristic epithelial cells of the tubular portion of the kidney are low columnar to cuboidal and rest on a thin basement membrane which is supported by a sheet of fibroblasts and a few smooth muscle fibers (Fig. 44). These epithelial cells thus border on the venous and sinus systems and are, therefore, in intimate contact with a rich supply of blood. The epithelial sheet of this portion of the kidney shows an irregular wavy appearance in cross section. The cells are somewhat variable in appearance. In well-fixed material the nuclei are usually round or oval, very rich in chromatin granules and vesicular in type (Fig. 44). They are usually located near the peripheral zone. The cytoplasm is vertically striated from the superior surface to the basement membrane. At times a few small vacuoles may be present in the cytoplasm near the lumen. Again, the vacuoles may be numerous, coalesce to form larger vacuoles, and occupy about two-thirds of the cell. Thus, the nuclei may be forced toward the basement membrane and the acidophilic striations then become delicate, displaced, and confined to the basal third. It is possible that these striations are intracellular canaliculi. The vacuoles contain variable amounts of PAS-positive materials.

The ureter is lined with the same type of epithelial cells as the tubular portion except at the area about the opening where the cells show a transition to the epithelium of the mantle cavity.

Saccular portion of the kidney. The wall of the saccular portion forms many prominent folds which run with the long axis of the kidney. The folds may reach the opposite wall. The epithelial cells of the wall of the saccular portion are columnar and are taller than those of the tubular portion but are arranged in the same manner. The folds consist of two epithelial sheets with a blood space between them (Fig. 45). The nuclei are round to oval and are basal in position. In each epithelial cell there is usually a large vacuole which frequently contains a crystalline concretion. The vacuole usually pushes most of the cytoplasm to the basal third of the cell. Unlike the cytoplasm in the tubular portion, the cytoplasm of the cells in the saccular

portion contains coarse acidophilic granules and filamentous material. Little PAS-positive material is present in the vacuoles.

The crystalline concretions were thought by Baecker (1932) to be the urine-substance in land pulmonates. They do not take ordinary stains. They are lightly refractile, round bodies with a central core and appear yellowish-brown in hematoxylin-eosin preparations (Fig. 45). In fresh specimens the presence of numerous concretions gives a yellowish-orange color to the saccular portion of the kidney. The concretions are very weakly PAS-positive.

The lumen of the saccular portion is connected with the pericardial sac by the "renopericardial canal." This canal has been described in *Helix* by Nüsslin and in *Arion* and *Limax* by von Rolle (Baecker, 1932). The canal in *Australorbis* is lined by a sheet of acidophilic cuboidal epithelium at the opening to the pericardial sac. These gradually transform to tall columnar cells toward the renal opening. The free surface of these cells is heavily covered with long cilia (Fig. 46). In specimens prepared in alcoholic fixatives, such as Newcomer's or Carnoy's, and stained with thionin, a few mucous cells may be observed scattered among the epithelial cells.

The mesenchymatous tissue in the wall of the saccular portion of the kidney, which forms part of the wall of the pericardial sac, has been described in the section dealing with amoebocytes.

VIII. Alimentary System

The histology of the alimentary system in pulmonates has been described in detail by Baecker (1932) and von Haffner (1923) for *Helix* sp. and by Carriker and Bilstad (1946) for *Lymnaea stagnalis appressa*. Baker (1945) described the gross anatomy of the alimentary system of *Australorbis glabratus* and Marcuzzi (1950) studied its histology. In general, this system in *A. glabratus* is comparable to that of *Helix* or *Lymnaea*, but with certain variations.

Morphologically and functionally, the alimentary system of *A. glabratus* can be divided into two parts: the digestive tract and the glandular organs. The digestive tract includes the

buccal mass, the esophagus, the stomach and the intestine. The glandular organs comprise three glands: the buccal, the salivary, and the so-called "liver."

The general structure of the digestive tract is the same throughout, with variations as to size and shape.

The lumen is lined with a sheet of simple columnar epithelium which rests on a basement membrane. The basement membrane is supported by two layers of smooth muscle fibers, an inner longitudinal and an outer circular layer. The thickness of each layer varies considerably. A sheath of connective tissue envelopes the outer muscle layer in the region where the digestive tract lies free in the body cavity.

Lips and oral cavity. The oral cavity is bordered externally by three lips, two upper and one lower, located in front of the buccal mass. In cross section, three lips form a "T"-shaped space, which with the buccal mass comprises the oral cavity. The marginal epithelium is composed of ciliated, tall columnar cells like those on the foot surface. These cells are gradually replaced toward the buccal mass cavity by cuticular cells. The cuticular layer which appears bluish-gray and homogeneous in hematoxylin and eosin preparation thickens toward the buccal mass and is transformed into three wedge-shaped horny jaws at the margin of the buccal mass (Fig. 47). The horny jaws stain orange to orange-pink in H-E preparations and appear striped, each stripe arising from individual epithelial cells. Beneath the epithelial sheet is a distinct basement membrane resting on thick dense connective tissue. Delicate smooth muscle fibers run in various directions in the connective tissue. In it are also embedded many mucous cells as well as a group of sensory cells (Fig. 32). A small amount of brown pigment may be observed rarely in the epithelial cells.

Buccal mass. The buccal mass, called the "pharynx" in *Helix* by Baecker (1932), functions primarily as a scraping and swallowing organ (Fig. 2). In addition to the complicated muscular layers it contains three distinct structures: i) the chitinous radular ribbon, ii) the radular sac, and iii) the radular carrier. The histology of the muscle of this organ has been described in an early paragraph (see Muscular Tissue). The epithelial

sheet which covers the oral cavity consists of tall columnar cuticular cells except on the floor where interrupted by the radular carrier. The cuticular cells contain centrally located oval nuclei which are rich in coarse chromatin granules (Fig. 48). Many acidophilic striations are present in the slightly basophilic cytoplasm, and these run from the basement membrane to the cuticular layer. They contain larger amounts of coarse granules of brown pigment than do the epithelial cells of the lips. The pigment granules are usually located in the zone between the cuticular layer and the nuclei. The cuticular layer appears bluish-gray in H-E preparation, is strongly positive with PAS-stain, and decreases gradually in thickness toward the esophagus where it is replaced by cilia. A group of vesicular cells which resemble those of the radular carrier is embedded in the dorso-anterior wall of the buccal mass and probably serves as an elastic cushion (Fig. 49). These cells will be described in a later paragraph.

Radula. The radula, a structure of taxonomic value, is a serrated chitinous ribbon originating in the radular sac. It passes forward on the floor of the buccal mass toward the lower lip and terminates near the horny jaw. The radular ribbon is supported by the odontophoral cartilage (or radular carrier) which acts as an elastic cushion to control its movement along with the radular protractor and retractor muscles. The radula consists of two parts, the serrated chitinous dentins, and a thin basal plate. The histology of the radula of *Australorbis* is essentially similar to that of *Helix* (Baecker, 1932). The dentins are acidophilic and are embedded in the thin, homogeneous basal plate which is lightly basophilic. A thin layer of connective tissue binds the radular ribbon to the dorsal surface of the radular carrier.

Radular sac. The radular sac is located at the ventro-posterior portion of the buccal mass near the junction of the buccal mass and esophagus. In cross section, it is roughly horseshoe-shaped with the convex side facing ventrally (Fig. 50). It is partially embedded in the musculature of the buccal mass and, together with the latter, is covered by a common connective tissue sheath. The radular ribbon is on the periphery and follows the outline of the horseshoe, being held between two sheets of epithelial cells. Both sheets appear to be an extension and modification of the

epithelial sheets of the buccal cavity. The epithelial cells between the radular ribbon and the outer connective tissue sheath consist of a layer of columnar cells (Fig. 51) which, in *Lymnaea stagnalis,* were called the "subradular epithelium" by Carriker and Bilstad (1946). These cells contain central, ovoid nuclei and basophilic cytoplasm and apparently give rise to the basal plate of the radular ribbon. The boundary between the subradular epithelium and the basal plate is largely obscured. Acidophilic striations occur in the cytoplasm in the zone between the nuclei and the basal cell membrane. No basement membrane is present. The other epithelial sheet (the supraradular epithelium) consists of cuboidal or low columnar cells, with more or less central, round nuclei (Fig. 51). These cells also contain rich basophilic granules and apparently give rise to the serrated dentins. Carriker and Bilstad (1946) described syncytium formation of these cells in *Lymnaea,* but the cell boundaries of the supraradular epithelium in *Australorbis* are clearly apparent.

The core or center of the radular sac is filled with a special type of supportive tissue, the collostyle (Fig. 50). Baecker (1932) described this particular tissue in *Helix* as "*Gallertgewebe*" and Carriker and Bilstad (1946) refer to it in *Lymnaea* as a gelatinous-like supportive tissue. The cells of the collostyle are irregular in shape and size, being round, ovoid, elongated or spindle-shaped and measuring 10 x 4 microns to 64 x 24 microns. They are packed together like epithelial cells. The nuclei are relatively small, round or oval, and poor in chromatin. The cytoplasm is lightly acidophilic and homogeneous but may contain filamentous material at times. Little PAS-positive material was demonstrated in these cells, but they stain weakly with alcian blue 8 GS. Muscle fibers of the radular retractor join the collostyle at the "opening" of the horseshoe (Fig. 50).

Radular carrier. The radular carrier is frequently referred to as the odontophoral cartilage and is composed of three elements bound together by connective tissue to form the shape of a boat; the floor of the boat is on the dorsal side to support the radular ribbon. The three pieces of tissue also form an enclosed sac which is connected to the circulatory system by a large blood vessel (Fig. 53). Since the radular carrier appears to have a pumping action,

and since an accessory heart has been reported in other molluscs (Michelson, 1956), the relationship of the lumen of the radular carrier with the circulatory system in *A. glabratus* suggests the possibility that it serves as an accessory heart in addition to possessing a supportive function. Histologically, the radular carrier is composed of vesicular cells and muscle fibers. The muscle fibers are the same type as those in the wall of the buccal mass, and run vertically to the surface of the radular carrier. The vesicular cells are polygonal, measure 70 x 30 microns and usually have relatively small nuclei (7 microns) which are eccentric and poor in chromatin (Figs. 18, 52). The cytoplasm is enclosed in a conspicuous cell membrane and contains a network of fine acidophilic filaments and some amorphous material. Both the fibrillar network and the cloudy material are strongly stained with PAS technic. Binucleated cells are sometimes seen. The vesicular cells in the antero-dorsal wall of the buccal mass are smaller than those in the radular carrier, but otherwise are similar. Both the inner and outer surfaces of the radular carrier are covered with thin connective tissue sheaths (Fig. 52).

The nature of the vesicular cells and their functions have been discussed by Baecker (1932) and are thought to be primitive cartilage cells (the prototypes of chondrocytes) and to serve as an elastic cushion for the radular ribbon.

Esophagus. The esophagus originates at the dorso-posterior wall of the buccal mass and runs posteriorly, parallel to and along the right side of the genital tracts. It joins the crop in the vicinity of the albumen gland. The ciliated simple epithelium is arranged in several longitudinal folds (Fig. 54). These folds clearly differentiate the esophagus from the postintestine where the folds are circular (Fig. 60). The epithelium consists of tall, uniform columnar cells (33 microns in a snail of 14.8 x 5 mm.) throughout the entire length of the esophagus (Fig. 55). They contain basal oval nuclei which are very rich in chromatin. The cytoplasm is filled with basophilic granules and may contain several vacuoles. The external surface is covered with long dense cilia (6 microns). Mucous cells or goblet cells are usually not found among the epithelial cells but do occur beneath the basement membrane in the muscle layers. The goblet cells measure 10 microns at the largest diameter, are tear-drop in shape and

contain relatively large nuclei (6 microns), rich in chromatin. In hematoxylin-eosin preparations these cells are difficult to recognize, but they stand out conspicuously in PAS-stained preparations. The goblet cells are morphologically distinct from buccal gland cells in that the former are smaller in size, are embedded singly among the muscle fibers and the cytoplasm is not basophilic. They secrete PAS-positive materials through the intercellular spaces of the epithelial lining.

Beneath the thick basement membrane are two layers of smooth muscle fibers; an inner longitudinal, and an outer circular layer. The muscle layers of the esophagus are thicker than those of the intestine. The longitudinal layer is thicker than the circular layer toward the buccal mass, but this characteristic is reversed near the crop.

Stomach. Baker (1945) recognized three anatomically distinct parts in the stomach of planorbids: 1) the crop, 2) the gizzard and 3) the pylorus.

Histologically, the crop and pylorus are similar. The simple epithelium of the two parts is composed of columnar cells (25 microns in an 18 x 5.5 mm. snail) and many goblet cells (Fig. 56). The chromatin-rich nuclei are oval and subbasal in position. The cytology of the epithelial cells does not differ materially from that of the esophagus except in size; the region also has many more goblet cells. Between the thin basement membrane and the outermost connective tissue sheath, there is a type of supportive tissue which does not resemble any other connective or supportive tissue observed in this snail (Fig. 56). It may attain 700 microns in thickness (18 x 5.5 mm. snail), but varies depending on the degree of distention of the organ. It resembles poorly differentiated mesenchymatous tissue with scarce cellular elements but is rich in collagenous-like fibers and ground substance. Two types of cellular elements are present in addition to smooth muscle fibers and amoebocytes. One of the cellular elements resembles a fibroblast and is more or less concentrated near the basement membrane. The other consists of irregularly shaped cells which vary considerably in size (4-18 microns). The nuclei of the latter cells are round or oval, usually eccentric in position and generally very poor in chromatic material. In hematoxylin-eosin preparations, the cytoplasm may appear vacuolated and

contain a few lightly acidophilic granules and brown pigment. The cell is, for the most part, obscure and difficult to recognize without special staining technics. The cell membrane stains green in Gomori's trichrome, appearing to enclose a vacuolated cytoplasm containing a nucleus. The vacuoles are filled with PAS-positive material in the form of granules or droplets. The ground substance is lightly basophilic, homogeneous and stains lightly with PAS technic. Collagenous-like fibers are lightly refractile and poorly stained in hematoxylin-eosin preparations but take a deep blue in Azan stain.

The smooth muscle fibers are not layered as in the intestine or esophagus, but run in various directions embedded in the ground substance of the supportive tissue.

The gizzard has the thickest muscular wall of the entire digestive tract and contains at least 10 layers at its middle portion. The inner surface is covered with simple columnar epithelial cells which measure up to 25 microns (18 x 5.5 mm. snail) and contain subbasal, oval nuclei. Except for the cuticular layer on the lumen surface of the epithelial cells of the gizzard these cells resemble those of the crop and esophagus. However, no mucus-secreting goblet cells were observed in the walls of the gizzard. Circular, longitudinal, oblique and radial muscle layers alternate in the wall, and weave a conspicuous pattern characteristic of the gizzard (Fig. 57). Cuticular epithelial cells have been observed in *Lymnaea stagnalis* (Carriker and Bilstad, 1946) and in *Helix* (Baecker, 1932) in the stomach. Many sand grains are usually present in the gizzard. Thus, the gizzard appears to serve primarily as a mechanical grinder of ingested foods.

Intestine. Baecker (1932) subdivided the intestine of *Helix* into *Duodenum, Blindsack* and *Enddarm.* We prefer, however, to subdivide the intestine of *A. glabratus* after the scheme used by Carriker and Bilstad (1946) for *Lymnaea stagnalis*, i.e., prointestine, midintestine, postintestine and cecum.

Prointestine. The prointestine makes a circular loop around the stomach after leaving the pylorus and is gradually transformed into the midintestine at the right side of the stomach. The epithelial sheet is most conspicuous in this portion of the intestine, occupies more than three-quarters of the intestinal wall, and displays an active secretory function. The epithelial

cells form a pseudostratified epithelium which may reach 88 microns in thickness in a snail of 18 x 5.5 mm. The nuclei are oval, are rich in chromatin, and lie at several levels (Fig. 57). The cytoplasm of some of these epithelial cells is filled with numerous basophilic granules, but in the majority it appears alveolar, the spaces being filled with a cloudy substance. Since this substance is strongly PAS-positive and is constantly discharged into the lumen of the intestine, it is likely that the prointestine is a secretory zone and corresponds to the glandular portion of the intestine of *A. glabratus* described by Marcuzzi (1950). The free surface of the epithelial cells is covered with dense short cilia (Fig. 58). The basement membrane is thin and difficult to discern. The muscular layers are also very thin and are composed almost entirely of circular fibers. The connective tissue sheath is relatively thick and consists of two to three layers of fibroblasts which may contain a few brown to brownish-black pigment granules.

Midintestine. The midintestine, beyond the prointestine, runs posteriorly along the dorsal side among the liver lobules, makes a "U" turn in the anterior third of the liver and continues anteriorly on the ventral side to become the postintestine near the pericardial sac. The intestinal wall is thinner in this portion than in any other area of the intestine including the esophagus. The epithelium is composed of the same type of ciliated columnar cells (18 microns in 18 x 5.5 mm. snail) as in the pylorus; however, there are more goblet cells (Fig. 59). There is no folding of this simple epithelium. The inner longitudinal muscle layer, almost absent in the wall of the prointestine, becomes more prominent in this portion.

Postintestine. The postintestine originates in the vicinity of the pericardial sac and is distinguished from the latter by circular folds which are formed by the epithelial sheet. It then enters the loose vascular connective tissue at the base of the central ridge (rectal ridge) and passes forward to the anus at the left rim of the pneumostome. The circular folds are conspicuous in longitudinal section and readily differentiate the postintestine from other portions of the digestive tract (Fig. 60). The columnar epithelial cells are taller than those of the midintestine and measure 42 microns in a snail of 18 x 5.5 mm.

(Fig. 61). The epithelial cells are similar in morphology to those found in the esophagus and the midintestine, but the mucus-secreting goblet cells are fewer than in the midintestine. The muscle layers shift their relative position as compared to the midintestine, so that the inner layer becomes circular and the outer layer longitudinal. Toward the anus (Fig. 62), the circular muscle fibers increase in number and form the anal sphincter. The basement membrane around the anus thickens considerably and contains much fibrillar material; some of the fibrils appear to run into the basal zone of the epithelial cells.

Cecum. The blind sac or cecum opens into the prointestine at its junction with the pylorus and shares a common opening with the hepatic duct. There are two low, opposing, longitudinal folds which, as in *Lymnaea stagnalis* (Carriker and Bilstad, 1946), delimit the incurrent and the excurrent tubules within the cecum. In addition to these folds the epithelial cells also form many fold-like circular elevations similar to those of the hepatic duct. Those cells covering the wall of the incurrent tubule, which is on the side of the hepatic duct, resemble the epithelial cells of the hepatic duct and possess a few goblet cells which secrete PAS-positive material. Those cells covering the excurrent tubule resemble the epithelial cells of the prointestine and actively secrete PAS-positive material (Fig. 63). Muscle fibers are scarce, but outer circular and inner longitudinal fibers are present. No basement membrane was observed. A thin connective tissue sheath covers the outer surface of the blind sac.

Buccal gland. Carriker and Bilstad (1946) studying *Lymnaea stagnalis,* claimed to have described the buccal gland for the first time. In *Helix pomatia,* this gland was called Nalepa's gland by Pacaut and Vigier (1906); however, since the cytology of the secretory cells could not be differentiated from that of the secretory cells of the salivary gland proper, Meisenheimer (1912) included it as part of the salivary gland. This group of gland cells in *Australorbis* is histologically distinct from the secretory cells of the salivary gland and should be identified as the buccal gland.

The major portion of this gland is located on the dorsal wall of the buccal mass, but extends to its ventral wall, as well as to a small portion of the esophagus. In fresh specimens the gland gives a characteristic yellow color to this area. The buccal gland

is composed of numerous mucus-secreting cells which form many small islands embedded among the muscular bundles of the buccal mass wall (Fig. 64). It is a ductless gland; the contractions of the surrounding muscle bundles apparently force the secretions through the intercellular spaces into the oral cavity. The individual secretory cells are usually pleomorphic but frequently they are flask-shaped. Large cells measure 15 microns at the base (in a snail of 18 x 5.5 mm.) when they are filled with PAS-positive material. The nuclei are round or slightly oval with chromatin practically filling the entire nucleus. The cytoplasm is strongly basophilic and also shows a positive reaction to PAS. However, it does not show metachromasia with toluidine blue or thionin. The buccal gland cells are similar to mucous gland cells in the foot and cannot be differentiated from the latter.

Salivary gland. The salivary gland of *A. glabratus* is a paired tubular organ, the distal ends of which are joined, thus forming a loop on the dorsal wall of the esophagus at its middle third. Each gland has a short duct which opens to the buccal cavity on the lateral aspect of the esophagus. The gland does not have an acinar structure as it does in *Lymnaea stagnalis* (Carriker and Bilstad, 1946), but the tubular wall bulges slightly to form haustra-like folds which resemble those of the vertebrate colon. No basement membrane was demonstrated in the epithelial sheet with the staining methods used. The epithelium rests on a thin connective tissue sheath which contains some dark brown pigment. Sub-epithelial smooth muscle fibers were described in *Helix* by Baecker (1932), but we did not observe them in *A. glabratus*. The ducts are short and lined with cuboidal, non-secretory epithelial cells that have long cilia on the surface. The nuclei are usually round, rich in chromatin granules and situated centrally. The cytoplasm is filled with acidophilic granules and may also contain a few vacuoles. The secretory cells gradually appear among the duct epithelial cells lying between the non-secretory cells in the basal zone. These secretory cells gradually increase in size and number toward the gland proper; they compress the duct epithelial cells and become the principal cell (Fig. 65). The flattened duct epithelial cells of the gland proper are very slender and are hardly recognizable

among the secretory cells except for their acidophilic, thread-like cytoplasm with a centrally located basophilic nuclear outline. The epithelial cells of the duct retain the long cilia and probably function as supportive structures for the secretory cells as well as propelling secreted materials (saliva) to the buccal cavity by the movement of the cilia. Pacaut and Vigier (1906), studying *Helix,* and Carriker and Bilstad (1946) studying *Lymnaea stagnalis,* differentiated several secretory stages in the secretory cells, but it was difficult to differentiate comparable stages in *Australorbis.* The actively secreting cells are tall columnar and measure 50 microns in height with round nuclei of 15 microns in an 18 x 6.0 mm. snail (Fig. 66). The nucleus is extremely rich in coarse chromatin, usually contains a large nucleolus and resembles the nucleus of a ganglion cell. Since cell function and regeneration are not synchronous, cells of various sizes are usually seen in a given area. The nuclei also are not of equal size and are located on two or three levels, *i.e.,* basal, central or subperipheral (Fig. 66). The cytoplasm is filled with a fine, basophilic reticular net which holds large amounts of secretory materials in droplets or coarse granules. These materials sometimes show light acidophilia, sometimes remain unstained in hematoxylin-eosin preparations, but are usually stained by PAS which frequently reacts strongly and masks the basophilic reticular net. Active secretion of the glandular cells apparently occurs by rupture of the cell membrane and thus the cell contents are discharged into the lumen of the gland (Fig. 66). Since degenerated nuclei of glandular cells frequently occur among the cell debris in the lumen, the secretory cells probably disintegrate completely after secretion (holocrine). Young secretory cells differentiate from fibroblasts in the connective tissue sheath. The cytoplasm of the differentiating fibroblast first thickens and becomes pyramidal, with the base of the cell resting on the connective tissue sheath. These young cells gradually grow larger and assume the morphology of columnar secretory cells with round, chromatin-rich nuclei; the cytoplasm accumulates a basophilic reticular net as well as PAS-positive material during the process.

Liver. The liver is a massive digestive gland and occupies the caudal two-thirds of the snail body together with the ovotestis; the latter is approximately one-fifth of the size of the liver. The liver is a compound tubular gland, consisting of a main hepatic duct with one short dorsal branch and numerous secretory lobules (Figs. 1, 5, 68). The main duct runs anteriorly along the ventral side of the lobules, passes into the cecum and thence via a common opening into the prointestine at its junction with the pylorus. The short, dorsal branch unites with the main duct shortly before its point of union with the cecum (Fig. 5). The simple columnar epithelium of the hepatic duct is raised in numerous fold-like, circular elevations which, in cross section, are dome-shaped, finger-shaped or fungoid in appearance (Fig. 67). These elevations decrease posteriorly in number and height and the epithelial sheet finally becomes smooth. The elevations are caused by different sizes of epithelial cells. Those in the elevated areas measure 25 microns in height and elsewhere measure 14 microns (in an 18 x 5.5 mm. snail). The epithelium of the raised areas near the opening of the duct into the cecum has a pseudostratified structure, but this is lost as the epithelium passes posteriorly. The free surface of the cells is covered with cilia. The cilia of each cell arise from a point near the nucleus, radiate to the surface, and penetrate through the basal bodies before becoming free in the lumen. This type of structure for ciliary apparatus has been observed in *Helix* by Baecker (1932). Mucus-secreting goblet cells are also present among the ciliated columnar cells. Except for the basal bodies of the cilia, the cytology of the epithelial cells of the hepatic duct is similar to that of the epithelium of the pylorus. Mitotic figures are occasionally seen in the epithelium of the hepatic duct. No basement membrane is recognized, and the epithelium is covered with a thin, connective tissue sheath which contains a few collagenous-like fibers. A few delicate smooth muscle fibers, mostly longitudinal, are seen between the connective tissue sheath and the epithelial sheet near the opening. The lobules of the liver are also covered with a thin, connective tissue sheath which is continuous with that of the hepatic duct. No basement membrane was observed in the lobules. Each lobule is embedded in and separated from its neighbor by a loose vascular connective tissue

which is continuous with that of the ovotestis. Numerous blood spaces, with varying numbers of vesicular cells and pigment cells, are present in the interstices of the connective tissue. The vesicular cells are similar to those described for the radular carrier and are perhaps storage-cells for polysaccharides. Von Brand and Files (1947) described storage of glycogen in the liver and ovotestis of *Australorbis glabratus* but did not specify where.

Faust (1920), Baecker (1932), and Carriker and Bilstad (1946) agree that only two types of cells (lime cells and digestive cells) occur in the liver lobules of the snails studied. In *Australorbis*, mucus-secreting goblet cells are present in addition to the digestive cells and lime cells. The goblet cells are sparsely scattered among the other two types of cells and cannot be differentiated from the young digestive cells except when PAS stain is used. They take a deep stain with leucofuchsin and are morphologically similar to those present in the other parts of the digestive tract. The lime cells are more abundant than the goblet cells and are usually pyramidal or rhomboidal in shape with their bases lying on the connective tissue sheath (Fig. 70). The larger cells measure 40 microns across (20 x 6.4 mm. snail) with relatively large, round nuclei (measuring 12 microns) which are extremely rich in chromatin granules and usually contain a large nucleolus. The cytoplasm is filled with basophilic granules and fibrillar reticulum; it may also include vacuoles of various sizes, but not inclusion bodies or the yellow excretory bodies of the type noted in the cytoplasm of the digestive cells (Fig. 68). The lime cells are embedded between digestive cells and usually do not reach the lumen surface except after breakdown of the latter in the secretory process. It has been observed that after the surface cell membrane ruptures, the nuclei may lose chromatin granules and separate from the cytoplasm. Therefore, it would appear that the secretory processes of the lime cells are holocrine in nature.

The digestive cells constitute the principal glandular epithelial cells and show considerable polymorphism. Krijgsman (1925) described four stages in the digestive cells of *Helix pomatia* and classified their secretory function as being apocrine in nature. Similarly four physiologic stages could be recognized

in the functioning of digestive cells of *Australorbis*. The digestive cells appear to break down completely during secretion (holocrine), and new cells arise or are differentiated from cells of the connective tissue sheath. The fibroblasts are first transformed into columnar cells which resemble the epithelial cells of the hepatic duct except that there are no cilia. The nuclei become round, are located subbasally and are rich in chromatin granules (Fig. 69). The cytoplasm is filled with basophilic granules and a reticular net. No inclusion bodies appear at this stage, and the lumen surface of the lobule is smooth (Fig. 69). In the second stage, the cytoplasm begins to accumulate secretory materials in the peripheral zone which is filled with coarse acidophilic granules (Fig. 59). Owing to the uneven accumulation of these acidophilic granules, the lumen surface of the lobule becomes irregular and cells become pleomorphic. In the third stage, vacuoles of various sizes appear in the cytoplasm peripheral to the nuclei which are now forced to a basal position (Figs. 68, 70). Yellow globular inclusion bodies, or so-called excretory bodies, are frequently found in the vacuoles, at first very small and several in number but later becoming larger in size and fewer in number. They are not stained in H-E preparations, assume their own yellow color, are lightly positive to PAS, and may also be stained with fast green (*gruene Granula* of Krijgsman, 1925 and 1929). The digestive cells reach their peak of growth at the third stage and measure 70 microns in height (20 x 6.4 mm. snail). At the fourth stage the cell membrane on the lumen surface ruptures, and the cell discharges its contents. The nuclei lose their rich chromatin granules, float free in the empty cells and finally disappear. The cytoplasm near the nucleus retains its basophilia until nearly replaced by vacuoles just prior to cell dissolution. The lumen of the lobules at the beginning of secretion is filled with cellular debris which transforms into an amorphous cloudy mass. This cloudy mass is lightly acidophilic and also stains lightly with PAS. Although the liver is directly connected with the intestinal tract, bacteria and other microorganisms are seldom seen in the hepatic lumen. Fedele (Baecker, 1932) studying "Opisthobranchium," ascribed secretory, phagocytic, absorptive and excretory functions to the

liver cells. We were able to obtain evidence only of secretory and possibly excretory functions in the cells of liver lobules in *A. glabratus*.

IX. Reproductive System

Since pulmonate snails may be identified by the characters of the reproductive system, the anatomy and histology of the reproductive organs have been studied heretofore in greater detail than those of the other systems (Baker, 1945; Hubendick, 1955). The anatomical descriptions of the reproductive organs of *A. glabratus* by Baker (1945) and Paraense and Deslandes (1955) are essentially similar except that the latter authors recognized the common collecting canal of the ovotestis as histologically different from the divertieula and hermaphroditie duct.

The genital system of *A. glabratus* can be subdivided into a) the common genital organs (hermaphroditie organs), b) male genitalia and c) female genitalia.

Common genital (hermaphroditic) organs. The common genital organs consist of a highly-branched ovotestis with its collecting canal and a hermaphroditic duct. The acini of the ovotestis are histologically and functionally distinct from the collecting canal. In spite of the morphological differences, Baker (1945) apparently regarded this collecting canal as part of the hermaphroditie duct. The cephalic or anterior portion of the collecting canal is expanded to form a pouch-like structure which abruptly narrows at its end to join the seminal vesicle or the origin of the hermaphroditic duct by a thin, "S"-shaped tube (Fig. 75). The hermaphroditic duct conveys mature male and female sexual cells into the vas efferens (sperm duct) and carrefour respectively.

Ovotestis. The walls of the acini of the ovotestis are composed of thin connective tissue, two to three cells thick, with abundant collagenous-like fibers. This wall, "Ancel's layer" (Ancel, 1902), contains cells which, in section, are morphologically similar to fibroblasts. Although Abdel-Malek (1954 a, b) in *Helisoma* and *Biomphalaria* and Merton (1930) in *Planorbis* described germinal epithelium with a basement membrane in the ovotestis, we observed no such specially differentiated layer in *Australorbis*.

It appears, however, that the germinal cells differentiate from the innermost cells of Ancel's layer by a thickening and transformation of the cytoplasm and nucleus. In section, the female germinal cells or ova are usually located at the apices of the acini, and the male germinal cells are arranged along the side walls (Fig. 71). The very early stages (spermatogonia) of the male germinal cells are frequently located near the atrium of an acinus. The maturing stages of the male cells generally line the wall of the acinus from the atrium toward the apex (Fig. 71), and the maturing spermatozoa attaching to the basal or Sertoli cells are close to the area where the ova are developing. The developing young male germinal cells are attached to Sertoli cells by cytoplasmic stalks, which in section frequently are not apparent, and thus some of the germinal cells appear to be free in the lumen (Fig. 90). The youngest male germinal cells are tear-drop in shape and measure 6 microns at the base. The chromatin-rich nuclei fill more than two-thirds of the cytoplasm which is also extremely rich in basophilic material. The spermatogonia separate by mitotic division to form spermatocytes which enlarge considerably before undergoing another division. The cytoplasm of the spermatocytes gradually loses its basophilia and becomes slightly acidophilic. Another mitotic division takes place (Fig. 72) resulting in the formation of spermatids. The spermatids are about the size of spermatogonia and half the size of the spermatocytes. The small round nuclei of the spermatids are at first compact, but before the transformation into spermatozoa is complete the chromatin material becomes concentrated and crescent-shaped (Fig. 89). The nucleus finally becomes helicoid, and the cytoplasm gives rise to an acidophilic tail portion or flagellum by elongation (Figs. 89, 90). Although meiosis must take place in the process of spermatogenesis, no special study was made of the reduction divisions in *A. glabratus*. The mature spermatozoon (Fig. 96) has a slightly flattened cephalic portion, shaped like a corkscrew, and a long flagellum with two loosely-wound spiral coils encircling the axis cylinder.

The Sertoli cells also differentiate from Ancel's layer at an early stage of spermatogenesis, but they remain inconspicuous until the spermatogonia reach the spermatid stage. The Sertoli cells vary in size and shape considerably, appearing as half-moon

or columnar-shaped. They contain one or occasionally two oval nuclei which are rich in chromatin, and contain a large nucleolus. A wedge-shaped constriction may occasionally be seen in the nucleus of Sertoli cells (Fig. 89). The cytoplasm is filled with a fine reticulum which may be acidophilic or basophilic. Vacuoles in varying number may be present in the Sertoli cells.

The female germinal cells are nearly always located at the apex of each acinus in small numbers, and also differentiate from the cells in Ancel's layer (Fig. 73). The mature ovum measures 98 microns in diameter and contains a round, slightly eccentric nucleus measuring 36 microns. The nucleolus is round, eccentric, and has a basophilic, half moon-shaped paranucleolus along the margin. The cytoplasm stains purple or bluish-purple in hematoxylin-eosin preparations, is coarsely granular, and may also contain a few small vacuoles. PAS-positive material may be present at times, as reported by von Brand and Files (1947). The nurse cells, which are equivalent to the Sertolis of the male germinal cells, adhere to the ovum to form an enveloping sac. A follicular cavity may occur between the nurse cells and ovum. Abdel-Malek (1954 a) described the presence of a follicular cavity in *Helisoma trivolvis*. Degenerating female germinal cells are frequently seen among the maturing ova.

Collecting canal. The collecting canal of the ovotestis is lined with a sheet of cuboidal cells which may have the appearance of transitional epithelium when the canal is not distended. No basement membrane is present, and the epithelial sheet is directly covered with a connective tissue sheath rich in collagenous-like fibers. A few delicate, smooth muscle fibers are present beneath the epithelium. The collecting canal is capable of great distention to accommodate the large numbers of germinal cells (mostly spermatozoa) produced at certain stages of reproduction. When the canal is distended, the epithelial cells become almost flat with the compressed nuclei occupying a central position. The epithelial sheet forms several longitudinal folds when the canal is not distended. The epithelial cells are then compressed and appear wedge-shaped with dome-like surfaces forming irregular wavy outlines in the lumen, and the oval nuclei are irregularly located at several levels, thus giving the epithelial sheet a transitional appearance (Fig. 74). Short cilia may be seen on these

cells. PAS-positive material can be demonstrated in the cytoplasm peripheral to the nucleus. The acidophilic cytoplasm is usually finely granular, containing a delicate reticulum.

Hermaphroditic duct or ovisperm duct. The hermaphroditic duct in *Australorbis* is histologically distinct from the collecting canal of the ovotestis and is not capable of great distention (Fig. 77). The duct joins the collecting canal via a small-calibre, "S"-shaped tubule (Fig. 75). Beyond this junction the hermaphroditic duct enlarges and possesses many diverticula which are usually filled with mature spermatozoa. This portion of the duct is generally called the seminal vesicle (Baker, 1945) (Fig. 76). The lumen of the duct, as well as the diverticula, is lined with simple cuboidal epithelium with central, round nuclei rich in chromatin. The epithelial cells in various diverticula may vary slightly in size. Contrary to the report of Paraense and Deslandes (1955), we have found the free surface of the epithelial cells to be covered with short, dense cilia. The cytoplasm is filled with lightly basophilic granules and may also contain vacuoles. Abdel-Malek (1954 a) described a syncytial form of the diverticular epithelium in *Helisoma*, but this character was not observed in *Australorbis*. The cytoplasmic boundary is particularly clear in preparations stained with Azan or Gomori's trichrome. Cilia are present throughout the entire duct. Beyond the seminal vesicle, the duct gradually narrows and becomes very small before joining the carrefour. Smooth muscle fibers are present beneath the epithelial sheet. A thin connective tissue sheath covers the outer surface. The duct joins the carrefour tangentially so that part of its wall is elevated from the surface of the carrefour (Fig. 97).

Male genitalia. Sperm duct or vas efferens. The male genitalia consist of the prostatic gland and genital tract. The genital tract is subdivided into sperm duct (vas efferens), vas deferens, and penial complex (Baker, 1945; Paraense and Deslandes, 1955). The prostatic gland is formed by evaginations of the sperm duct and possesses the same histological structure as the duct. Although the sperm duct in *Australorbis* is very important in the reproductive process of this hermaphroditic snail, the precise origin of the duct has not been described. This is probably due to the extremely small calibre of the duct at its origin, thus

making the exact location of its departure from the hermaphroditic duct difficult to find by gross dissection. Abdel-Malek (1954 a) showed that the sperm duct of *Helisoma* arises from the hermaphroditic duct just before the latter empties into the carrefour. The sperm duct in *Australorbis* originates from the hermaphroditic duct at its opening into the carrefour; thus both ducts in reality have a common opening into the carrefour. For a distance of approximately 200 microns from its origin, the sperm duct is about 33 microns across and is histologically distinct from the rest of the duct. The epithelium of this portion consists of low cuboidal, non-secretory cells covered with long, dense cilia (Fig. 78 a). The cytoplasm is acidophilic, and the nucleus is compact. The epithelial sheet is invested with many smooth muscle fibers, most of which are circular and constitute the inner layer. The muscle layers are covered with connective tissue which is common to the carrefour, the end portion of the hermaphroditic duct and the first portion of the oviduct. Continuing toward the penial complex, the epithelial cells of the sperm duct gradually increase in height to become columnar, and secretory cells appear among the non-secretory (Fig. 78 b). The secretory cells finally predominate and compress the non-secretory cells which become small and obscure (Fig. 81). Both types of cells alternate in the epithelial sheet. The secretory cells measure 52 microns (20.0 x 6.4 mm. snail), and have round to oval, chromatin-rich nuclei (7 microns) located in the basal zone (Fig. 81). The cytoplasm in actively secreting cells contains a basophilic reticulum, the meshes of which are filled with secretory droplets. These droplets appear first as fine granules but later coalesce and become droplets or globules. In hematoxylin-eosin preparations, the secretory granules stain blue or blue-purple, and the droplets stain pale blue or are refractory to staining. In PAS technic, the secretory granules stain weakly, and the droplets stain strongly. The non-secretory cells are as tall as the secretory and are ciliated. The cytoplasm is usually so compressed between the secretory cells that it appears as an acidophilic thread. The central nuclei are then so small and compact that only a blue oval outline can be distinguished. The epithelial sheet is invested with a few delicate circular, smooth muscle

fibers but is without a basement membrane. A thin connective tissue sheath containing some dark brown pigment covers the outer surface.

Prostatic gland. The prostatic gland is histologically similar to the glandular portion of the sperm duct and represents evaginations of the latter.

The vas deferens. The sperm duct tapers abruptly in the vicinity of the duct of the seminal receptacle and is transformed into the non-secretory vas deferens (Fig. 82). The vas deferens continues toward the praeputium, enters the tissue of the neck near the male genital opening and makes a "U" turn on the dorsal surface of the praeputium; then lies free in the coelomic cavity and joins the verge. Histologically, the portion of the vas deferens between the sperm duct and the "U" turn (proximal leg) is different from the portion between the "U" turn and the verge (distal leg). The columnar epithelial cells of the sperm duct are replaced abruptly by densely ciliated, non-secretory, cuboidal cells which rest on a thick basement membrane. The nuclei of the cuboidal cells are round, central in position and rich in chromatin granules. Circular muscle fibers which are sparse in the sperm duct become abundant. Longitudinal fibers do not appear in the outer layer until the proximal leg reaches the "U" turn. The proximal leg is narrower (66 microns at its origin and 33 microns near the "U" turn in a snail of 20 x 6.4 mm.), is usually oval in cross section, and contains few longitudinal muscle fibers (Fig. 83). The distal leg is thicker (uniformly 122 microns in a snail of 20 x 6.4 mm.), round in cross section, and the outer longitudinal muscle layer is as thick as the inner circular layer (Fig. 84). The muscle fibers of the distal leg, especially the longitudinal fibers, contain a large amount of PAS-positive material around the nuclei, and then appear vacuolated in cross section in hematoxylin-eosin preparations (Fig. 84). A thin connective tissue sheath covers the outer surface of the vas deferens.

Penial complex. The penial complex is composed of the verge, the vergic sac and the praeputium (Baker, 1945). The inner surface of the verge is lined with a layer of low cuboidal cells containing ovoid, central nuclei and bearing dense, long cilia.

The epithelial cells rest on a basement membrane which is almost as thick as the epithelial sheet. Beneath the basement membrane are two layers of muscle fibers, an inner circular and an outer longitudinal. A thin connective tissue sheath covers the outer surface (Fig. 85 a).

The vergic sac is lined with a sheet of nonciliated cuboidal cells. The basement membrane is invested with a layer of inner circular and outer longitudinal smooth muscle fibers. The outer surface of the vergic sac is covered with a relatively thick connective tissue sheath containing rich dark brown pigment. The sac is frequently seen in an invaginated position in the fixed specimen (Fig. 85 b).

The praeputium opens on the neck behind the base of the left tentacle and is characterized by a highly-developed muscular wall, especially in the region of the two opposing longitudinal ridges called pilasters (Fig. 86). The pilasters extend into the lumen which appears "H"-shaped in cross section. The simple epithelium consists of columnar cells with dense, long cilia. The nuclei are very rich in chromatin, ovoid in shape and subbasal in position. A thick basement membrane supports the epithelial sheet. Two layers of circular muscle fibers are present, one in contact with the basement membrane and the other in contact with the connective tissue sheath covering the outer surface. Between these two layers, there is a zone of connective tissue which is richly invested with both longitudinal and radial muscle fibers. The longitudinal fibers are separated into bundles by radial fibers and connective tissue (Fig. 86). The longitudinal bundles are especially well-developed in the pilasters. No mucous cells are present in the epithelial sheet, but they occur in the connective tissue, apparently discharging their secretions into the lumen via the intercellular spaces of the epithelial cells. Abundant small pigment cells are found in the connective tissue sheath and among the muscle fibers.

The region between the praeputium and the vergic sac is called the diaphragm (Baker, 1945). This is a muscular ring with papilla-like protrusions of epithelial folds into the lumen (Fig. 87). The epithelial cells are low columnar, bearing a cuticular covering on the surface and have round chromatin-rich

nuclei in the subbasal zone. An inner layer of circular and an outer layer of longitudinal muscle fibers lie beneath the basement membrane.

Female genitalia. The female genitalia include the albumen gland, carrefour, oviduct, nidamental gland, uterus, spermatheca (seminal receptacle) and vagina (Baker, 1945). The carrefour, oviduct, nidamental gland, uterus and vagina comprise a continuous duct which carries the mature ova to the exterior. The microscopic anatomy of the female genitalia follows a general scheme like that previously described in *Lymnaea stagnalis* by Holm (1946). The simple epithelial sheet contains two types of cells, a massive secretory and a slender supportive cell. These two cells alternate quite regularly to form the duct lining. There is usually no basement membrane beneath the epithelium. Delicate smooth muscle fibers are seen between the epithelium and the outer connective tissue sheath.

Albumen gland. This is a compound tubular gland situated on the dorsal side of the stomach. Two histologically distinct structures are present: a main excretory duct and a large number of highly branched secretory tubules. The main excretory duct opens into one pole of the carrefour which histologically is an enlarged end portion of the duct (Fig. 79). The simple epithelium of the duct consists of low columnar cells covered with long dense cilia. The cytoplasm contains acidophilic striations running vertically to the surface and a central, oval nucleus. The lumen is frequently filled with a cloudy, lightly basophilic secretion of the gland. No basement membrane was observed beneath the epithelium which is invested by inner longitudinal and outer circular smooth muscle layers. The connective tissue sheath covering the outer surface is continuous with that of the gland.

The epithelium of the secretory tubules contains two types of cells, a massive secretory and a slender supportive cell. The secretory cells vary considerably in size and structure depending on the phase of secretory activity. In a snail of 20 x 6.4 mm., they measure 12 x 7 microns as secretory globules begin to accumulate. The nuclei measure 5 microns, are round, central in position, extremely rich in chromatin, and contain a large nucleolus. The cells gradually enlarge and measure 30 x 22.5

microns just before the globules are discharged. The secretory materials appear first as fine granules embedded in an amphophilic (purple-red in H-E preparation), cytoplasmic reticulum (Fig. 88). They coalesce and form one or more large globules which are secreted into the lumen through the ruptured cytoplasmic membrane. The scanty cytoplasm which is forced to the basal region at this stage is highly basophilic and cannot be differentiated clearly from the darkly stained (pyknotic) nucleus. The globules stain a light blue in H-E preparations, blue in Azan, bluish-green in Gomori's trichrome, are strongly positive with PAS, but do not stain with thionin or toluidine blue O. The secretory materials in the lumen also react in a similar manner with these stains. Von Brand and Files (1947) reported that the galactogen was responsible for the PAS reaction in this gland. Millon's reaction was also applied with a moderately positive result. The supportive cells are usually compressed tightly between the massive secretory cells and show poorly defined cytology. The acidophilic cytoplasm appears as a thin thread containing an elongated blue nucleus in the center. The nuclei of the supportive cells may be displaced either toward the basal or the peripheral zone. In the latter case, they were described in land pulmonates by Cavalié and Beylot (1902) as "*Cellules centrotubuleuses,*" but in *Australorbis* are actually cells of a supporting nature, as described by Baecker (1932). The secretory tubules are covered by a thin connective tissue sheath. The tubules are separated by connective tissue containing small pigment cells and abundant blood spaces.

Carrefour. Baker (1945) called the bean-shaped swelling at the end of the albumen gland duct the "carrefour" (Fig. 97). It had earlier been described and considered to be the site of fertilization of the ova in hermaphroditic land pulmonates (Meisenheimer, 1907 and 1912). The hermaphroditic duct opens tangentially into the carrefour opposite the opening of the duct of the albumen gland. Macroscopically, it appears partially embedded in the wall of the carrefour. The oviduct opens on the side wall of the carrefour between the sperm duct and the duct of the albumen gland. Except that the epithelial sheet contains some mucous cells and is infolded, the structure of the carrefour cannot be distinguished from that of the duct of the albumen

gland and therefore, the carrefour should be regarded as the enlarged end portion of this duct (Figs. 79, 80). From its micro-anatomical and histological structures, it appears that the function of the carrefour in *Australorbis* is more likely a device for separating the male and female germinal cells into their respective genital tracts than a fertilization site as reported by Meisenheimer (1907) for *Helix pomatia*, and in *Helisoma* and *Biomphalaria* by Abdel-Malek (1954 a, b). Since this structure has not been described in dioecious snails, and since the sperm duct is extremely small in calibre at its opening to the carrefour and shares a common opening with the hermaphroditic duct, the spermatozoa are probably delivered to the sperm duct directly, while the ova, too large to enter the sperm duct, are passed to the carrefour to be delivered to the oviduct.

Oviduct. The oviduct originates from the carrefour, runs anteriorly in the coelomic cavity along the sperm duct and expands into the nidamental gland as a pouch-like swelling in the vicinity of the posterior end of the prostatic gland (Baker, 1945) (Fig. 97 e). The epithelial cells of the oviduct are differentiated from those of the carrefour by their glandular structure, basophilic staining, and the absence of dense cilia. The lumen of the duct is folded irregularly. The secretory cells are massive and columnar measuring 87 microns in height (15 x 5.1 mm. snail) and contain relatively small compact nuclei in the basal zone (Figs. 79, 80, 91). The cytoplasm is filled with a basophilic, fine reticulum, containing amorphous secretory material. The secretory material stains strongly with alcian blue and PAS, and shows metachromasia with thionin (beta metachromasia). These reactions indicate the presence of acid mucopolysaccharides. The non-secretory cells were called sustentacular cells by Paraense and Deslandes (1955), and are tightly compressed between the secretory cells. They are poorly differentiated and usually appear as light acidophilic threads containing thin, central, ellipsoidal nuclei. Contrary to Paraense and Deslandes (1955), we found the sustentacular cells to be sparsely covered with long cilia on the surface. Like those of the sperm duct, the cells are apparently supportive structures for the oviduct. No basement membrane was observed and the epithelial

sheet is invested directly with a few delicate smooth muscle fibers. A thin connective tissue sheath covers the outer surface.

Nidamental gland. The anterior end of the oviduct is enlarged considerably and is twisted into a half circle to form a pouch-like structure called the "nidamental gland" by Baker (1945) (Fig. 98). Its lumen is almost completely occluded by irregular epithelial folds. The secretory and sustentacular cells are the same size as those in the oviduct; however, the staining reactions of the secretory cells are strikingly different (Fig. 91). Over a small area facing the spermatheca the epithelial sheet contains only non-secretory, low columnar, acidophilic cells (about one-fourth of the height of secretory cells) possessing abundant cilia. These cells are also sustentacular in nature and are not modified by the pressure of the secretory cells. The slender sustentacular cells seen in the oviduct are also present among the secretory cells of the nidamental gland. The majority of the secretory cells are filled with acidophilic secretory globules which press the small, compact oval nuclei to the basal zone. These globules are strongly stained with PAS and show purple metachromasia with thionin (beta metachromasia) but are not stained by alcian blue, thus indicating a different chemical nature from the secretory substance in the oviduct. A small number of the secretory cells stain lightly basophilic in H-E preparations and lack secretory globules. Their cytoplasm is filled with cloudy material containing fine granules, which stain positively with PAS but show much weaker metachromasia with thionin than do the acidophilic cells. The cloudy material does not stain with alcian blue. The smooth muscle fibers are minimal in the wall covered by the secretory cells but are more abundant in the wall covered only by the low columnar sustentacular cells.

Uterus. The uterus is a short segment of the female genital tract that connects the nidamental gland to the vagina. It is not differentiated macroscopically from the nidamental gland except for its wider lumen. Histologically, differences are apparent in the epithelial sheet and in the muscle layer (Fig. 92). The secretory cells in the uterus resemble the basophilic secretory cells of the nidamental gland. At the area of transformation from the nidamental gland, the secretory cells are approximately the same size as those in the latter, but gradually become smaller

toward the vagina. The cytoplasm is reticular and contains basophilic secretory material. This material is PAS-positive but is negative with alcian blue and shows no metachromasia with thionin. The compact nuclei are oval and are located between the mid- and basal zones. The non-secretory sustentacular cells are more abundant and less tightly pressed between the secretory cells than those in the nidamental gland. These cells become very numerous and finally replace the secretory cells completely to transform into vaginal epithelium (Fig. 93). Cilia are abundant owing to the increasing number of sustentacular cells. The non-secretory area of the epithelium in the nidamental gland is continuous with the uterus and ends at the junction of the latter with the vagina. No basement membrane is present beneath the epithelium. The muscle fibers become more numerous toward the vagina and form inner longitudinal and outer circular layers. The outer connective tissue sheath contains small pigment and mucous cells.

Vagina. The vagina is the only segment in the female genital tract wherein the epithelial sheet rests on a basement membrane. The irregular epithelial folds of the uterus are rearranged into several longitudinal folds. The epithelial sheet is composed of non-secretory, heavily ciliated, columnar cells, among which occur a few mucus-secreting goblet cells. The cytoplasm is acidophilic, containing oval nuclei in the basal zone. The inner longitudinal muscle fibers become less abundant, while the outer circular fibers become very well developed toward the female genital opening. This aperture is located between the male genital opening and the anus. The lumen usually appears occluded by the long cilia and the epithelial folds. The outer connective tissue sheath is fused with the surrounding tissue of the neck and contains abundant small mucous cells (Fig. 95).

Spermatheca or seminal receptacle. The spermatheca is a pear-shaped sac which opens into the vagina at its middle and posterior thirds. The short duct of the spermatheca is similar histologically to the vagina. The heavily ciliated, simple columnar epithelium of the duct forms several longitudinal folds and rests on a relatively thick basement membrane (Fig. 94). The inner longitudinal and outer circular muscle layers beneath the basement membrane are well developed. The anterior half of the

duct is embedded in connective tissue in common with the vagina and a portion of the proximal leg of the vas deferens. The cilia of the epithelial cells are long and dense near the opening but become shorter and more sparse toward the sac epithelium and finally disappear on the latter. The longitudinal muscle fibers of the duct run into the spermatheca. The epithelium of the sac is composed of columnar cells which decrease in size from the duct to the end of the sac. Many folds are present when the sac is empty. The columnar cells become cuboidal when the sac is distended. The chromatin-rich nuclei are round and located in the basal zone. The cytoplasm is filled with a fine basophilic reticulum and may occasionally contain several small vacuoles (Fig. 85). The cytoplasm superficial to the nucleus stains with PAS but does not take up alcian blue or show metachromasia with thionin. The epithelial cells apparently possess secretory functions. The spermatozoa in the spermatheca may occasionally lose their structure completely and appear degenerate.

SUMMARY AND CONCLUSION

A histological study of *Australorbis glabratus* has been carried out as a necessary requisite for subsequent histopathological investigations on this snail. The results are presented as a systematic description of nine organ systems or tissues. It is believed that this study represents one of the first of such descriptions of a fresh-water snail, although similar descriptions exist for the land pulmonates.

The epidermal tissue consists of simple epithelium except for a small area of the mantle collar where pseudostratified epithelium is present. The epithelia of the foot surface, the tentacles and a small portion of the mantle cavity are heavily ciliated.

Two types of connective tissue are recognized: a) the dense connective tissue containing abundant fibroblasts, pigment cells, collagenous-like fibers and ground substance but lacking conspicuous circulation spaces; b) the loose "vascular" connective tissue characterized by the presence of numerous circulation spaces formed by a meshwork of fibroblasts. The fibroblasts appear to be an important cellular element owing to their ability

to transform into amoebocytes under certain stimuli and to repair damaged tissue.

The muscular tissue contains three types of muscle fibers: a) the granular muscle fibers, confined to the heart; b) the intermediate granular muscle fibers found only in the buccal mass; c) the smooth muscle fibers composing the rest of the muscular tissue of the other organs. The structure of the heart muscle in *A. glabratus* differs from that of the land pulmonates which possess obliquely striated fibers.

Eleven ganglia forming a ring around the esophagus comprise the central nervous system. These ganglia are connected to one another by commissures. The ganglion cells are arranged peripherally, and the nerve fibers centrally, in each ganglion. The statocyst, which is imbedded in the epineurium at the dorsoposterior corner of each pedal ganglion, is lined with two types of epithelial cells and contains a number of ovoid statoliths. The eyes are well developed and structurally are composed of the optic capsule, cornea, retina, lens, vitreous humor and optic nerve. The osphradium is a pear-shaped sac enveloped at its base by the osphradial ganglion and is located at the junction between the mantle collar and the neck on the left side of the median line. This organ has not been described in land pulmonates and is believed to be a sense organ present only in aquatic snails. The tentacle has a dense connective tissue core containing a central artery, and has peripheral blood sinuses in the region between the core and the epithelial sheet. There are five groups of conspicuous peripheral sensory cells, one at the base of each tentacle and one at the margin of each lip.

The heart is provided with two valves, the atrioventricular and aortic valves. No endocardium was observed, but the epicardium is well defined. The only type of blood cell, the amoebocyte, is probably formed under normal conditions in lymphoid tissue located in the wall of the kidney, and in the blood sinuses. The lymphoid tissue has not been reported (in the pulmonate) prior to this study and in appearance resembles the medulla of a lymph node in the vertebrates.

The respiratory surface is covered with four types of epithelial cells: flat, cuboidal, columnar without cilia, and ciliated columnar. The last type of epithelial cells has not been reported in the land pulmonates.

Unlike *Helisoma* sp., the renal organ of *A. glabratus* consists of two distinct parts, an anterior tubular and a posterior saccular portion. The lumen of the kidney is connected with the pericardial sac by the renopericardial canal which is lined with heavily ciliated columnar cells.

The alimentary system consists of a digestive tract and three digestive glands. The digestive tract is covered for the most part with a simple, ciliated epithelium containing a number of goblet cells and is provided with two layers of smooth muscle fibers. The buccal gland comprises a group of mucous cells which can not be distinguished morphologically from the cells of the foot mucous gland. The salivary gland is composed of a pair of simple tubular organs with haustral-like folds in the gland proper and is morphologically different from that in *Lymnaea* sp. or in the land pulmonates where a compound tubular structure has been reported. The holocrine, simple glandular epithelium secretes PAS-positive material, which is delivered to the buccal cavity via the ducts. The liver is a compound tubular gland which delivers its secretion to the prointestine via the main hepatic duct. The simple, glandular epithelium has no basement membrane and contains two types of cells, the digestive and lime cells. The hepatic ducts are provided partly with circular, fold-like elevations which are formed by epithelial cells of varying heights.

In contrast to the findings in *Helisoma* sp., *Biomphalaria* sp. and the land pulmonates, the acini of the ovotestis of *A. glabratus* are lined with cells which can not be differentiated morphologically from fibroblasts and do not possess a basement membrane. The common collecting canal of the ovotestis is provided with a transitional epithelium and is capable of great distention. The mature germinal cells in the common collecting canal are delivered via the hermaphroditic duct to the carrefour where they are separated into the respective genital tracts. The carrefour, which has been found only in the hermaphroditic snails, is histologically an enlarged end-portion of the main duct of the albumen gland in *A. glabratus*. The vas efferens and prostatic gland are histologically similar and are the glandular portions of the male genitalia. The vas deferens, verge, vergic sac and praeputium are muscular structures and are considered devices

for copulation. The albumen gland, oviduct, nidamental gland and uterus are the glandular portions of the female genitalia. The vagina is a muscular tube and serves primarily as a copulatory organ.

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EXPLANATION OF PLATES

Magnifications of photomicrographs and drawings are indicated in the appropriate legends for the figures. Fixatives and staining technics used for each figure are also given. Small letters indicating various detail structures are independent for each figure. Drawings were made with the aid of a camera lucida. All figures were reduced to ¾ on reproduction.

The abbreviations used in the legends are as follows: Zenker: formic acid—Zenker's fixative; Newcomer: Newcomer's fixative; PFF: formic acid—Bouin's fixative; Methyl: absolute methyl alcohol; H-E: hematoxylin-eosin; H-Phlox: hematoxylin-phloxine B; H-Az H-E: Hexatoxylin-azure II-eosin; PAS: periodic acid—Schiff reaction; Gomori: Gomori's trichrome; Azan: Mallory's trichrome; Fresh: fresh, unfixed and unstained specimen.